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THE BLOOMING OF WHEAT FLOWERS[1]

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INTRODUCTION

The time and manner of blooming of wheat flowers is the result of the interaction of internal and external factors. When flowers have reached the blooming stage, the exact time and rate of blooming is determined to a large extent by meteorological conditions.

The observations on wheat flowers reported in this paper were made primarily for the purpose of procuring additional information on the effect of external factors on blooming.

METHODS AND MATERIALS

The plants on which most of the observations were made were volunteers growing in a garden in Washington, D. C. They were of the Fultz type (*Triticum vulgare*), having the following characters: Plant, winter habit; spike, awnless; glumes, glabrous, white; kernels, red, soft. More than 400 flowers in seven heads on four plants, which began to bloom at about the same time, were chosen for observation. Additional observations were made on plants growing in the field and in the greenhouse at Arlington Experiment Farm, Rosslyn, Va.

A plane view diagram of a wheat head illustrated by figure 1 shows the method used for recording the data. In figure 1, the rectangular spaces inclosed by solid lines represent the odd-numbered spikelets found on one side of a head, numbering from the base of the head, and the rectangular spaces inclosed by dotted lines represent the even numbered spikelets found on the opposite side. The spikelet number is shown within the large square located in the middle of each rectangular space. The numbers in the small squares adjoining the large square indicate the alternate position of each flower on the spikelet from the lowest to the highest. The exact time of blooming was recorded in the spaces opposite the flower number.

Flowers blooming cleistogamously were recorded as "dehisced inside." Blank spaces indicate sterile flowers. On spikelets 6, 7, 12, and 13, of head No. 4A (see fig. 1), fifth flowers bloomed but were not included in the data.

The seven heads chosen were watched continuously for six days, with the exception of a short period each night between 10 p. m. and 1.55 a. m. when little active blooming occurred, and the exact time of blooming of practically all flowers was recorded. Observations at night were made with the aid of a small electric flash light which was directed on the flowers at various intervals long enough only to procure the necessary information for record.

[1] Received for publication, Dec. 13, 1923.

	4	22	3	3:23 A.M. 4/18	
6:15 P.M. 4/15	2		1	4:30 P.M. 4/14	
12:35 A.M. 4/17	3	21	4		
6:09 A.M. 4/14	1		2	7:42 A.M. 4/14	
8:15 P.M. 4/16	3	20	4		
4:43 P.M. 4/14	1		2	4:47 P.M. 4/15	
10:23 A.M. 4/18	4	19	3	5:32 P.M. 4/16	
4:19 A.M. 4/15	2		1	DEHISCED INSIDE	
2:43 P.M. 4/16	3	18	4	9:08 P.M. 4/18	
DEHISCED INSIDE	1		2	1:47 P.M. 4/15	
4:00 A.M. 4/13	3	17	4		
12:10 P.M. 4/16	1		2	12:45 P.M. 4/15	
2:25 P.M. 4/16	3	16	4	3:23 A.M. 4/18	
9:10 A.M. 4/15	1		2	10:26 A.M. 4/15	
11:30 A.M. 4/17	4	15	3	9:35 A.M. 4/16	
DEHISCED INSIDE	2		1	9:38 A.M. 4/15	
DEHISCED INSIDE	3	14	4	11:30 A.M. 4/17	
9:59 A.M. 4/16	1		2	9:30 A.M. 4/15	
9:35 P.M. 4/16	4	13	3	Below 8:30 P.M. 4/16 and 3:55 A.M. 4/18	
9:38 A.M. 4/15	2		1	8:55 A.M. 4/16	
	3	12	4	11:59 A.M. 4/17	
10:28 A.M. 4/16	1		2	10:26 A.M. 4/15	
12:06 P.M. 4/17	4	11	3	2:18 P.M. 4/16	
12:28 P.M. 4/15	2		1	Below 8:30 P.M. 4/16 and 2:55 A.M. 4/17	
3:22 P.M. 4/16	3	10	4	4:59 A.M. 4/18	
5:33 A.M. 4/14	1		2	4:34 P.M. 4/15	
4:30 P.M. 4/16	3	9	4	4:09 A.M. 4/18	
8:32 A.M. 4/15	1		2	6:08 P.M. 4/15	
7:10 P.M. 4/16	3	8	4	8:05 A.M. 4/18	
11:10 A.M. 4/15	1		2	8:36 A.M. 4/16	
10:16 A.M. 4/18	4	7	3	1:30 A.M. 4/17	
10:02 A.M. 4/16	2		1	3:46 A.M. 4/15	
2:33 P.M. 4/18	4	6	3	4:08 P.M. 4/18	
3:03 P.M. 4/16	2		1	Below 10:00 P.M. 4/15 and 2:43 A.M. 4/16	
5:25 A.M. 4/18	3	5	4	Below 8:30 P.M. 4/16 and 3:55 A.M. 4/18	
6:30 A.M. 4/16	1		2	4:30 P.M. 4/14	
12:30 P.M. 4/18	4	4	3	10:32 A.M. 4/18	
11:40 A.M. 4/17	2		1	10:08 A.M. 4/16	
5:35 P.M. 4/18	3	3	4	6:30 P.M. 4/18	
5:06 P.M. 4/14	1		2	4:00 A.M. 4/18	
11:25 A.M. 4/18	3	2	4		
11:51 A.M. 4/17	1		2	7:29 A.M. 4/18	
	4	1	3	DEHISCED INSIDE	
DEHISCED INSIDE	2		1	12:05 P.M. 4/17	

FIG. 1.—Diagram of wheat head No. 4A, showing the method used for recording the data on time of blooming of the different flowers.

Immediately after recording the time of blooming of a flower, the empty extruded anthers were removed with forceps in order to facilitate observations and to prevent confusion in recording the data. Great care was required in this procedure since the slightest jarring or touching of a head while blooming is likely to hasten the opening of the flowers. A flower was considered as having bloomed as soon as noticeable separation of the lemma from the palea occurred.

THE BLOOMING PROCESS

Under favorable conditions the wheat flower blooms by opening its glumes, slowly at first, then more rapidly, until the tips of the lemma and palea are separated, usually 3 or 4 mm. After the glumes open the anthers are pushed upward by the elongating filament and when fully extruded assume a pendent position. At some time during these processes the anthers dehisce apically along the lines joining the cylinders into pairs. This splitting may take place in one or more anthers by the time they are first clearly visible, while in others it may be delayed until the time of full extrusion. A number of flowers were observed whose anthers were completely extruded and pendent before dehiscence occurred. Some flowers were found which had retained within their glumes one, two, or three anthers, quite often lodged in the folds of the palea. Other flowers were found with anthers partially protruded and imprisoned between the tips of the glumes. The greatest length of filament observed was 10 mm., exclusive of the anther, which was 3 mm. long. In one instance a filament was observed to attain its full length of 10 mm. in 10 minutes. Askenasy (*1*)[2] measured a number of filaments of wheat and rye flowers. He states that in most cases the filaments have grown from 1 to 1½ mm. per minute.

Very soon after full extrusion, and assisted by the inversion of the anthers, the pollen is fully emptied from the anther sacs. Some of it, probably at least one-third, usually falls within its own flower, resulting in plentiful pollination of the stigma. The remaining pollen is scattered about and may fall upon the stigmas of other flowers. Cross pollination apparently is sometimes effected in this way, especially when the anthers of neighboring flowers have been removed, as shown by Leighty and Hutcheson (*8*), or have aborted.

When conditions are unfavorable for the opening of the glumes, the anthers of the wheat flower shed their pollen and effect fertilization without being extruded, or they may be extruded only at the tips. Nearly 5 per cent of the flowers, or 19 out of a total of 406, under observation in this study, behaved this way. Each of the heads under observation had one or more such cleistogamous flowers, the maximum number on any head being six. Kernels were procured from all of these flowers. Under certain environmental conditions wheat flowers may be quite generally cleistogamous.

TIME REQUIRED

The process of blooming of wheat flowers is variable. Some flowers have been observed to open in less than 1 minute. Others require 3 minutes or more. Observations on the time elapsing between the beginning of opening and the time at which the anthers attain the

[2] Reference is made by number (italic) to "Literature cited," p. 244.

pendent position following their complete extrusion from the glumes were made on 25 flowers at different hours of the day favorable for blooming. The time required for the anthers to assume the pendent position varied in these 25 flowers from 1 minute 40 seconds to 5 minutes 25 seconds, and averaged 3 minutes 36 seconds. The time required depends on (1) the position of the head at the time of the protrusion of the anthers, (2) the resistance offered by the glumes, (3) air movement, (4) temperature, and (5) rainfall and humidity.

The time elapsing between the opening and closing of the glumes of a flower was found to vary from 11 to 66 minutes. Frequently difficulty was experienced in determining the exact time when the glumes were closed completely, for sometimes anthers or filaments became lodged obscurely between the glumes in such a way that complete closing was prevented. Table I shows the duration of blooming of 25 flowers, with their location on the spike. The average duration was found to be 26.5 minutes.

TABLE I.—*Time of opening and closing and time elapsing between opening and closing of 25 flowers, with head number, position of spikelet on the head and position of the observed flowers on the spikelet*

Head number.	Spikelet number.	Flower number.	Time of opening.	Time of closing.	Time between opening and closing.
			1922	1922	*Minutes.*
2 D	17	1	5.55 p. m., May 14	6.18 p. m., May 14	23
3 C	10	1	5.28 p. m., May 14	5.41 p. m., May 14	13
3 C	16	1	5.29 p. m., May 14	5.40 p. m., May 14	11
4 A	22	1	6.15 p. m., May 15	6.36 p. m., May 15	21
4 A	22	2	4.50 p. m., May 14	5.20 p. m., May 14	30
4 A	17	2	12.45 p. m., May 15	1.10 p. m., May 15	25
4 A	11	2	12.28 p. m., May 15	12.50 p. m., May 15	22
4 A	12	2	10.26 a. m., May 15	10.45 a. m., May 15	19
4 A	20	1	4.43 p. m., May 14	5.10 p. m., May 14	27
4 A	10	1	5.33 p. m., May 14	6.15 p. m., May 14	42
5 C	15	1	5.07 p. m., May 14	5.40 p. m., May 14	33
5 C	11	2	2.25 p. m., May 15	2.45 p. m., May 15	20
5 C	13	1	5.07 p. m., May 14	5.40 p. m., May 14	33
5 C	5	1	1.39 p. m., May 16	2.15 p. m., May 16	36
5 C	8	2	8.21 a. m., May 16	8.50 a. m., May 16	29
5 C	14	1	5.30 p. m., May 14	5.50 p. m., May 14	20
6 C	13	1	5.30 p. m., May 14	5.55 p. m., May 14	25
7 D	7	1	7.49 a. m., May 16	8.55 a. m., May 16	66
7 D	18	2	8.34 a. m., May 16	9.15 a. m., May 16	41
8 B	17	1	10.27 a. m., May 15	10.45 a. m., May 15	18
8 B	13	1	9.30 a. m., May 15	10.00 a. m., May 15	30
8 B	16	1	10.27 a. m., May 15	10.48 a. m., May 15	21
8 B	12	1	8.13 a. m., May 15	8.29 a. m., May 15	16
8 B	10	1	10.27 a. m., May 15	10.48 a. m., May 15	21
8 B	8	1	10.27 a. m., May 15	10.48 a. m., May 15	21
Average					26.5

Kirchner (5) and Koernicke and Werner (7) state that under favorable conditions wheat flowers remain open for about a quarter of an hour. According to Knuth (6) both Rimpau and Delpino found that each flower remains open only about 15 minutes. Früwirth (2) states that the flower is closed usually in 12 to 20 minutes, sometimes 8 to 35 minutes, from the beginning of opening. Obermayer (9) records the duration of

blooming under most favorable conditions as 13 to 18 minutes, but states that it sometimes is completed in shorter periods, even as low as 2 or 3 minutes, while under less favorable conditions and in the early morning it requires from 25 to 32 minutes. The average duration is 22 minutes.

Percival (*10, p. 122-129*) states that the time taken in opening and closing completely varied from 8 to 30 minutes or more, the average being 20 minutes, while Hays (*4, p. 50*) says, "The floret (of wheat) usually opens about dawn, and closes again within an hour."

PERIOD OF BLOOMING

Flowers were observed blooming during various hours of daylight and darkness. "Day" is hereafter referred to as a 24-hour period; "daylight," the period from sunrise to sunset; "twilight," the combined periods of approximately 1¾ hours each before sunrise and after sunset. "Night" includes the remaining portion of a day not previously designated. The division of the day into these periods was based on the time of sunrise and sunset (Table II) kindly supplied by the United States Naval Observatory at Washington, D. C.

Of the 406 flowers observed, 6.9 per cent bloomed at night; 6.9 per cent during twilight, and 86.2 per cent in the daylight. The total number of flowers blooming on each head and the numbers blooming in each period of the day during the complete period of anthesis are shown in Table III.

TABLE II.—*Time of rising and setting of the sun at Washington, D. C., May 14 to 19, 1921*

	May 14.	May 15.	May 16.	May 17.	May 18.	May 19.
Sunrise, a. m.	4.56	4.55	4.54	4.54	4.53	4.52
Sunset, p. m.	7.12	7.13	7.14	7.15	7.16	7.17

TABLE III.—*Total number of flowers blooming and numbers blooming during stated periods of the day, during the complete period of anthesis, on each of 7 heads of wheat*

Head No.	Number of flowers blooming.			
	Total.	In daylight.	In twilight.	In night.
2 D	59	52	4	3
3 C	52	42	4	6
4 A	75	62	9	4
5 C	41	37	1	3
6 C	51	47	2	2
7 D	53	47	3	3
8 B	75	63	5	7
Total	406	350	28	28
Per cent	100	86.2	6.9	6.9

The fact that most of the flowers under observation bloomed in daylight does not mean, necessarily, that light is essential for blooming. As shown elsewhere in this paper, wheat flowers kept in a dark room bloomed completely and in the same manner as flowers under natural conditions. Although no data are at hand, it is probable that night

blooming might be considerably more abundant than it was in this experiment under certain environmental conditions.

The effect on blooming of the duration of the daily illumination period, or of changes in the duration of this period, was not determined. It is known from other studies, however, that many varieties of wheat will bloom and mature in less time after planting when subjected to a long daily illumination period as compared with a shorter period.

According to Godron (*3*) wheat flowers open at 4.30 a. m. when the temperature is at least 16° C., with blooming ending at 6.30 to 7 o'clock. Rimpau (*11*) states that under favorable conditions blooming begins at 4.30 a. m. and occurs during various periods of the day. He reports that he observed open flowers after 6.45 p. m., but does not mention the latest hour at which blooming was observed. Koernicke (*7*) states that open flowers may be observed at all hours of the day, the earliest around 5.45 a. m., the latest at 8.30 p. m. Früwirth (*2*) observed flowers blooming during the day from 4.30 a. m. to 7 p. m. under favorable conditions. Obermayer (*9*) found that blooming began even before 5 a. m. and continued throughout the day, with a small number blooming after 7 p. m. Salmon (*12*), describing conditions in western South Dakota, says: "It is exceptional to find wheat in bloom after 7 a. m. under normal conditions."

ORDER OF BLOOMING

Table IV shows the positions on the head of the first five flowers blooming on each of the seven heads studied. On five of the seven heads the first flower bloomed in the lower half of the uppermost third of the head; on one head, in the upper half of the middle third; and on another head, in the upper half of the uppermost third.

Früwirth (*2*) states that the first blooming occurs between the middle of the head and the top of the middle third. Percival (*10, p. 122–129*) says that the position of the first flower to bloom is generally in the middle third of the head, usually in the upper part. Godron (*3*) found that the first flower bloomed below the upper third of the head. Rimpau (*11*) states that the first flower began to bloom at a point two-thirds to three-fourths of the distance from the base of the head.

Table V shows the position of the last flower observed to bloom on each head. On heads not included in this study flowers in the terminal spikelet have occasionally been seen blooming before those in spikelets located in the upper third of the head.

TABLE IV.—*Number of spikelets in each of seven wheat heads and position of the spikelets, numbered in order from the base of the spike, in which were located the first five and the last blooming flowers, with flower number of last flower*

Head No.	Total number of spikelets.	Position number of spikelets where occurred blooming of first five and last flowers.						Number of last flower.
		1st.	2d.	3d.	4th.	5th.	Last.	
2 D	20	16	14	15	20	17	5	4
3 C	18	13	15	14	12	11	3	3
4 A	22	20	22	10	21	9	3	4
5 C	18	12	15	13	11	14	9	3
6 C	19	16	14	11	12	13	4	3
7 D	21	15	17	14	13	16	8	3
8 B	23	15	12	11	13	9	2	2

Considering the head as a whole, the flowers above the first flower to bloom completed their blooming before those situated below this flower. From 8 to 60 per cent of the flowers below remained unopened at the time those above had finished blooming.

The lowest flower in the spikelet usually blooms first, and the other flowers of the spikelet bloom in order from lowest to highest on successive days. A few exceptions were found, however, where flowers failed to behave in this manner (see fig. 1, head 4A, spikelets 11, 12, 13, and 17). A number of instances were found where two flowers in the same spikelet bloomed on the same day. Complete data on these flowers is shown in Table V. In all, 39 such instances were recorded. The average time which elapsed between the blooming of the two flowers in the spikelet was nine hours and four minutes.

TABLE V.—*Location, by spikelet and flower number, of two flowers in the same spikelet which bloomed on the same day, with the hour of each blooming and the interval between these bloomings*

Head No.	Spikelet No.	Flower number and hour of blooming.				Interval between bloomings.
		1st.	2d.	3d.	4th.	
						Hr. Mm.
2 D	5	8.45 a. m.	8.30 p. m.			11 45
	6	7.52 a. m.	2.42 p. m.			6 50
	6			8.27 a. m.	5.45 p. m.	9 18
	10		8.20 a. m.	9.15 p. m.		12 55
	11	8.43 a. m.	3.45 p. m.			7 02
	18	6.45 a. m.	6.12 p. m.			11 27
3 C	8	10.16 a. m.	7.55 p. m.			9 39
	9	9.15 a. m.	4.29 p. m.			7 14
4 A	5	8.30 a. m.	4.30 p. m.			8 ..
	6	4.08 a. m.	2.33 p. m.			10 25
	8		8.56 a. m.	7.10 p. m.		10 14
	9	8.12 a. m.	6.08 p. m.			9 56
	16	9.10 a. m.	10.26 a. m.			1 16
5 C	6	8.30 a. m.	3.13 p. m.			6 43
	8		8.21 a. m.	9.40 p. m.		13 19
	10	6.49 a. m.	3.35 p. m.			8 46
	16	8.24 a. m.	6.45 p. m.			10 21
6 C	6	9.23 a. m.	5.10 p. m.			7 47
	9		7.56 a. m.	8.28 p. m.		12 32
	10	9.18 a. m.	3.53 p. m.			6 35
	13			3.09 p. m.	2.35 p. m.	11 26
7 D	3	4.47 a. m.	8.30 p. m.			15 43
	6	8.49 a. m.	5.07 p. m.			8 18
	7	7.49 a. m.	4.17 p. m.			8 28
	12	11.12 a. m.	8.31 p. m.			9 19
	13		7.34 a. m.	5.07 p. m.		9 33
	14	7.51 a. m.	5.43 p. m.			9 52
	16	8.02 a. m.	4.52 p. m.			8 50
8 B	5	8.09 a. m.	5.03 p. m.			8 54
	6	9.23 a. m.	3.50 p. m.			6 27
	8		9.44 a. m.	8.26 p. m.		10 42
	9	9.30 a. m.	6.17 p. m.			8 47
	10	10.27 a. m.	5.15 p. m.			6 48
	11	9.30 a. m.	5.15 p. m.			7 45
	12	8.13 a. m.	2.38 p. m.			6 25
	13	9.30 a. m.	5.15 p. m.			7 45
	14	10.20 a. m.	5.15 p. m.			6 55
	16	10.27 a. m.	6.48 p. m.			8 21
	21	9.42 a. m.	8.50 p. m.			11 08

Percival (*10, p. 122–129*) states that the lowest flower of a spikelet blooms first, with the others following on successive days. Früwirth (*2*) notes the exception that the second flower, instead of blooming on the next succeeding day, may sometimes bloom in the afternoon of the first day, while the remaining flowers may bloom on the following day or on the second day after the blooming of the second flower.

More than half of the first or lowest flowers in all the spikelets on the seven heads under observation were observed to bloom before the second flower in any spikelet bloomed (Table VI), and about 85 per cent of the first flowers bloomed before any flower in the third position bloomed. Nearly two-thirds of the second flowers in turn bloomed before the third flower in any spikelet bloomed, and a similar relationship exists in the blooming time of the third and fourth flowers. If the earliest blooming of second, third, and fourth flowers in any spikelet on a head is considered as marking off periods of blooming for that head, it is found that a part of the first or lowest flowers bloom subsequently to the opening of the earliest of the third flowers and even the earliest of the fourth flowers. Similarly, some of the second and third flowers will bloom after the first blooming of third and fourth flowers, respectively. The data on these periods in the heads under observation are shown in Table VI.

TABLE VI.—*Relative period of blooming of the first to fourth flowers in the spikelets of seven wheat heads*

Relative period of blooming.	Percentage of flowers in each position blooming in each period.				
	1st.	2d.	3d.	4th.	All.
Previous to the first blooming of a second flower	*Per cent.* 54. 13	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.* 17. 74
From the blooming of the earliest second flower to the blooming of the earliest third flower	30. 83	64. 40	.....	[a] 1. 79	29. 06
From the blooming of the earliest third flower to the blooming of the earliest fourth flower	10. 53	27. 97	69. 70	.....	28. 57
Subsequent to the blooming of the earliest fourth flower	4. 51	7. 63	30. 30	98. 21	24. 63
	100. 00	100. 00	100. 00	100. 00	100. 00

[a] One flower only; disregarded in period determinations.

The numbers of flowers that bloomed at the different hours of the day and night throughout the entire blooming periods of the seven wheat heads are shown in Table VII. Since the actual time of blooming of flowers was not observed between 10 p. m. and 1.55 a. m., all of the flowers blooming between these hours are grouped and recorded as of this period. The maximum number of flowers blooming on a single head in one day was 25. This occurred on May 16, one of the most active blooming days in the period of observation. Obermayer (*9*) observed a maximum of 23 flowers on one head opening in one day.

The maximum number of flowers blooming on a single head in one hour in this study was found to be eight, these being divided equally between the two sides of the head. The blooming on all heads was completed within 101 to 123 hours.

TABLE VII.—*Number and distribution per head of wheat flowers observed in the actual process of blooming between stated hours on each day of the period of anthesis at Washington, D. C., 1922.*

Blooming period.	May 14.									May 15.									May 16.									May 17.									May 18.									May 19.									Hourly total.
	Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.	All heads.		Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.	All heads.		Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.	All heads.		Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.	All heads.		Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.	All heads.		Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.	All heads.	
A. M.																																																			
2.01 to 3																																																			7
3.01 to 4																			1								1								1	2	3		3	1	1	1	3	15									17
4.01 to 5																																				1			4			2		7									7
5.01 to 6																1	1																			1			1		1	1		4									6
6.01 to 7										1	1			1			3																																			8
7.01 to 8															1	2	3		1	1	1		1	3		7										1			1		2	1		5						1	1		15
8.01 to 9										1			1	2			4		3	1	3	3		4		14										2			1	1	1			7									32
9.01 to 10										1	2	4	2	2	1	3	15		1	1	2	1	4		8	17										1								1									32
10.01 to 11											2		2		1	1	5	11	1			2			1	4					1			1		2	3	3				3	14							1	1	32
11.01 to 12												1			1	1	3				1				2	3		3	1	7	1	3		2	17	1	1					1	3		1	1	1	1	1	1	6	32
P. M.																																																		
12.01 to 1												2					2				1					1		1		2	1		3	4	11	1				1			2			1				1	17
1.01 to 2										5	4	1	3	3			16		1	1		2				4																2								1	21
2.01 to 3		1	4					5		1	2		1		1		5		7	4	3	4	7	3		28		1						1							1	2								41
3.01 to 4		1	1			1		3		2		1	3	5	3	1	15				3	2	1	1	2	9																									27
4.01 to 5		1		2	1			4		2	2	3	1	1	3	1	13			2	2		1	2	4	11									1				1			2									30
5.01 to 6		2	2	1	4	6		15							1	6	7		2	2	2	1	2	5	4	18									1							2									42
6.01 to 7		1		1				2		1		2	1	1		2	7			1			1			2		1						1															1		13
7.01 to 8											2						2				1					1									1	1																4
8.01 to 9															1		1		1				1		2	4																2									7
9.01 to 10																			1		1	1		1		4									1	1																5
10 p. m. to 1.55 a. m	1						1	2		1	1					1	3								2	2											1	3	1	1		2	8									15
Daily total	7	7	4	5	7	1		31		14	16	18	14	14	17	22	115		19	13	22	14	18	19	25	130		4	3	9	2	3	5	8	34		13	12	19	5	8	10	18	85		2	1	3	1	1	1	2	11	406

METEOROLOGICAL RELATIONS

The meteorological data used in this study are those for Washington, D. C., and were procured from the United States Weather Bureau. That temperature, rainfall, and sunshine are intimately associated with the blooming of wheat flowers appears evident from a consideration of figure 2. Temperature, however, seems to be the most important of the meteorological influences affecting blooming. The two days showing the most significant relation between temperature and flower blooming are May 15 and May 16, when almost perfect sunshine with no rainfall was registered. On these two days 60.8 per cent of all the flowers bloomed. Maximum blooming occurred on May 15 and 16, when the temperatures were 76° and 78° F., respectively. Active blooming usually begins shortly after sunrise, on days with clear skies, and anthers in flowers opening on these days dehisce more rapidly than those in flowers opening on cloudy days. Cloudiness or rain has the effect of retarding blooming, principally through the lowering of the temperature to a point below the optimum for this process. On cloudy or rainy days, however, when the temperature is favorable for blooming the flowers either open incompletely or bloom cleistogamously. On May 18 flowers were observed opening in a drizzling rain and pollen was shed from the anthers. No flowers were observed to open at any time during a rain of such intensity as to permit droplets of water to adhere to the glumes.

Three periods of intense blooming were noted: (1) On May 14 between 5 and 6 p. m., (2) on May 15 between the hours of 9 to 10 a. m., 1 to 2 p. m., and 3 to 4 p. m., and (3) on May 16 between the hours of 9 to 10 a. m., 1 to 3 p. m., and 5 to 6 p. m. The weather conditions on May 15 and 16 were very similar, and it is interesting to note the consistent correspondence of the three periods of intense blooming on these two days. The single period of intense blooming on May 14 apparently corresponds to the third period on May 15 and 16. On both the latter days a larger number of flowers bloomed in the second or middle period than in the first or third periods, which are of approximately the same intensity. The interval between the first and second blooming periods of these two days is approximately twice as great as between the second and third periods.

Früwirth (*2*), of Austria, states that when the temperature at 4.30 a. m. is above 14° C. the blooming begins at this hour. Many flowers bloom from this time to 5.30, while from 5.30 to 9 the number is less. Very many bloom from 9 to 10 a. m., while very few bloom from 10 a. m. to 2.30 p. m. From 2.30 to 3.30 p. m. the number again is large, while from 3.30 to 7 p. m. it again is small. The principal blooming time of the morning, made up of a preceding and succeeding period of which the first or the second may be the stronger, is followed in the afternoon by a later blooming period which approaches in intensity the weaker part of the morning blooming time.

On May 17, when lower temperatures prevailed, the only period of intense blooming was between 11 a. m. and 12 m., while on May 18 two periods occurred, one from 3 to 4 a. m. and one from 10 to 11 a. m. The intense blooming on May 14, 15, and 16 exhausted the flowers available for blooming to such an extent that with unfavorable atmospheric conditions on the 17th and 18th (cloudiness, rainfall, fluctuating temperature) only irregular blooming occurred on these days.

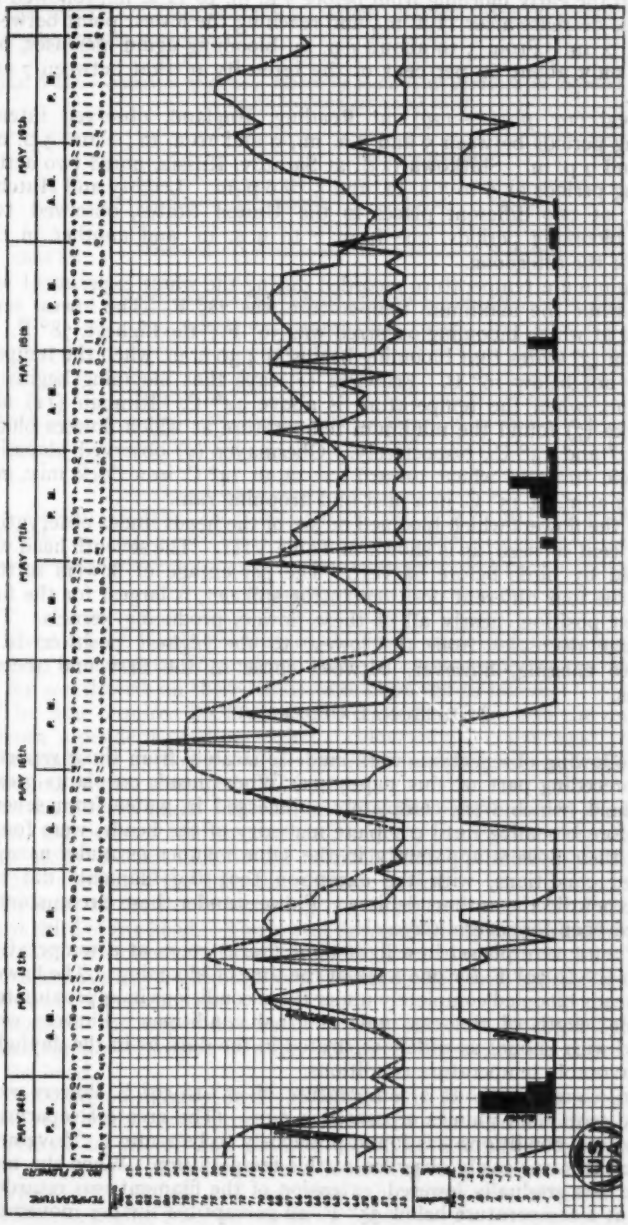

FIG. 2.—Temperature, rainfall, and sunshine record and number of flowers blooming on seven wheat heads at different hours in the period from 2 p. m., May 14, to 10 p. m., May 19, 1922, at Washington, D. C.

Obermayer (*9*), in Germany, reports that the most active blooming occurs in the early morning from before 7 a. m. to 11 a. m., followed by a marked decrease after 11 a. m., and reaching the lowest point between 1 and 3 p. m. From 3 to about 7 p. m. blooming again increases, but reaches only about 60 per cent of the intensity of that between 7 and 11 a. m.

Percival (*10. p. 122–129*), at Reading, England, observed intense blooming periods between 5 and 7 a. m., 9 and 10 a. m., 2 and 3 p. m., and 8 and 9 p. m. Shitkowa (*13*), at Saratov, Russia, noted two active blooming periods at 5 to 7 a. m. and 5 to 6 p. m. Leighty and Hutcheson (*8*), at two different places in the United States, observed two intense blooming periods, one from 7 to 9 a. m. and another in the middle of the afternoon.

The minimum temperature recorded during the whole experiment was 55° F., while the maximum temperature was 78° F. The lowest temperature at which blooming occurred was 56° F.; the highest, 78° F.

Godron (*3*) states that blooming begins at 4.30 a. m. when the temperature is not below 16° C. Früwirth (*2*) says that blooming begins at 4.30 a. m. when the temperature is above 14° C. Rimpau (*11*) and Koernicke (*7*) found the minimum temperature at which flowers bloom to be 12° and 13° C., respectively. Obermayer (*9*) observed blooming in a single strain of wheat toward 6 a. m. at 14° C. in a thick mist and toward 7 a. m. at 15° C. in a mist and dropping rain.

Of all the flowers which bloomed on six of the heads under observation 80.2 per cent set seed, as is shown in Table VIII. The seventh head was destroyed by birds before harvest. The percentage of flowers setting seed in the four different positions in the spikelet, is largest for the first or lowest position, nearly all of these flowers producing kernels. The percentage decreases from the lowest to the highest position, little difference existing, however, between those in the first and second positions.

ADDITIONAL OBSERVATIONS

The following observations were made separately from those reported in the preceding part of this paper: (1) Wheat heads on plants grown in the field, which were completely submerged in water from several days before blooming until complete maturity of the heads, were found to open their flowers apparently in the same manner as under normal, unaltered conditions, with the exception that the filaments did not elongate nor did the anthers dehisce. Flowers under these circumstances remained open for several days.

(2) Plants were placed in a light-tight (dark) room at a temperature between 60° to 70° F. for one week at the time of blooming. The flowers on the heads bloomed completely under these conditions in approximately the same length of time as under normal conditions. Flowers were observed with glumes as widely separated in the dark as in the daylight, under favorable temperature conditions.

(3) In a greenhouse at a temperature of 55° to 56° F. flowers were observed with their glumes separated 3 mm. (The greatest separation under normal conditions was found previously to be 4 mm.) Movement of the anthers was easily observed at this temperature. When the temperature was gradually lowered, extension of the filament was retarded, so that at a temperature below 55° F. no perceptible further movement

was detected, and the anthers appeared to remain stationary, but pollen was discharged at as low a temperature as 52° F.

The maximum temperature at which flowers were observed opening in a greenhouse was 80° F.

TABLE VIII.—*Numbers of flowers blooming and kernels produced in different positions in the spikelets on six wheat heads*

Head No.	Location in spikelet.									
	1st flower.		2d flower.		3d flower.		4th flower.		Total.	
	Number of—		Number of—		Number of—		Number of—		Number of—	
	Flowers blooming.	Kernels obtained.	Flowers blooming.	Kernels obtained.	Flowers blooming.	Kernels obtained.	Flowers blooming.	Kernels obtained.	Flowers blooming.	Kernels obtained.
2 D	18	17	18	18	16	14	9	5	61	54
3 C	17	16	17	17	15	12	7	3	56	48
4 A	22	22	22	21	21	15	16	2	81	60
6 C	17	16	17	17	13	12	5	2	52	47
7 D	19	19	20	19	11	10	6	0	56	48
8 B	23	22	23	19	18	9	13	0	77	50
Total	116	112	117	111	94	72	56	12	383	307
Per cent	.....	96.6	.....	94.9	.....	76.6	.....	21.4	.....	80.2

SUMMARY

Seven wheat heads growing in the open were under practically continuous observation throughout their blooming periods, and nearly all of the 406 flowers on these heads were observed to bloom.

The time required for a wheat flower to open fully and the anthers to assume a pendent position varied considerably, but averaged 3 minutes 36 seconds for 25 flowers, while the time from beginning of opening to complete closing averaged 26.5 minutes.

Of all flowers under observation, 86.2 per cent bloomed during daylight, 6.9 per cent during twilight, and 6.9 per cent during the night. This study did not include the determination of the effect on blooming of the duration of the daily illumination period.

In most of the heads blooming began in the lower half of the uppermost third of the head. The lowest flower in the spikelet usually bloomed first and the others followed in order from lowest to highest, usually on successive days, although two flowers in the same spikelet sometimes bloomed on the same day.

Some of the upper flowers in certain spikelets bloomed before the lowest or lower flowers in other spikelets, depending on their position in the head.

Blooming on different heads extended over periods from about 101 to about 123 hours, a maximum of 25 flowers on one head blooming in a day.

Periods of intense and reduced blooming alternated throughout the day, the time of their occurrence depending to a considerable extent on temperature, rainfall, and sunshine. Blooming was observed at temperatures ranging from 56° to 78° F., inclusive.

The percentage of seeds set in the spikelet decreases from the lowest to the highest position, little difference existing, however, between the first and second positions.

In additional experiments flowers were observed to open without dehiscence of the anthers when submerged in water. Also, full and apparently normal blooming occurred in continuous darkness. Temperatures of 55° F. and below checked blooming in certain flowers, although pollen was discharged from anthers of flowers previously opened when the temperature was reduced as low as 52° F.

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THE EFFECT OF SODIUM HYDROXID ON THE COMPOSITION, DIGESTIBILITY, AND FEEDING VALUE OF GRAIN HULLS AND OTHER FIBROUS MATERIAL[1]

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The aim of this investigation has been to improve the digestibility and feeding value of grain hulls and similar fibrous material. The agency used for this purpose has been sodium hydroxid, and its effect in varying concentrations on five different substances has been studied. The substances are oat hulls, barley hulls, rice hulls, cottonseed hulls, and flax shives.

It is of interest to note here that the annual output of oat hulls by three of the leading oat-milling concerns in the United States totals over 100,000 tons.[3] Although at the present time this by-product is mixed with the oat middlings and dust, which are also by-products of the mills, and is marketed as "oat feed," the product is admittedly of inferior feeding value, due to its high content of indigestible fiber. Any method the employment of which will bring about a considerable increase in digestibility of this and similar by-products is worthy of investigation. Aside altogether from their possible significance in a practical way, the facts brought out by the investigation are of considerable scientific interest.

Originality is not claimed for the method employed in the work. It was devised by Dr. Ernst Beckmann, of Berlin, Germany, for the purpose of hydrolyzing straw, and has been patented by him both in Germany and in the United States (*3*).[4] However, a careful search of the literature reveals that while considerable investigation has been carried on with straw, work with hulls has never before been attempted. The writer has studied the action of dilute sodium hydroxid as it affects the proximate and, to a certain extent, the ultimate composition of grain hulls, and has fed the untreated and treated hulls to sheep, ascertaining by the usual procedure of digestion experiments the effect of the alkali on the digestibility of the hulls.

REVIEW OF THE LITERATURE

The review has been arranged under three headings: The chemistry of fibrous material; the action of sodium hydroxid and other alkalies on fiber; development of the process of hydrolyzing fibrous material for feeding purposes, together with the results of feeding experiments with the various products.

These different phases of the problem overlap more or less and some investigators have dealt with all three of them, but each forms a sufficiently clear-cut division to warrant dealing with them individually.

[1] Accepted for publication Nov. 24, 1923.

[2] This investigation was made under the direction of J. B. Lindsey. It was begun by C. L. Beals, who made a partial study of oat and rice hulls, and to whom due credit is given. It is published with the permission of the director of the Massachusetts Agricultural Experiment Station, and constitutes the review and experimental data of a thesis to be presented in partial fulfillment of the requirements for the degree of master of science in the graduate school of the Massachusetts Agricultural College.

[3] From approximate estimates furnished by the manufacturers.

[4] Reference is made by number (italic) to "Literature cited," p. 263–265.

THE CHEMISTRY OF FIBROUS MATERIALS

The amount of work which has been done on the chemistry of plant fibers is nothing short of monumental, and yet, despite the vast accumulation of information now available, the chemistry of cellulose and of its combinations with pentosans, lignin, and allied substances in woody fiber is but imperfectly understood. Investigators are still at variance over the structure of the cellulose and the lignin molecule.

The writer has made no attempt to review all or even any considerable portion of the literature on the subject. A great deal of it is of a highly technical nature and of importance to the paper industry rather than to agriculture. It will be sufficient to outline the general concept of the chemistry of fiber as it exists at the present time, with such references as may be necessary.

Plant fibers are simply an aggregation of the cell walls of certain specialized cells occurring in the plant. These cell walls become elaborated, enlarged, and strengthened with age until maturity is reached, at which time, in high-fiber plants at least, they constitute the major portion of the individual cells and of the vegetative portion of the plant, the protoplasm having been almost, if not altogether, absorbed, or transported to the seed.

In the early stages of growth the cell wall is known to consist of practically pure cellulose, but with advancing age it becomes changed to a compound cellulose known as ligno-cellulose, which is characteristic of all fibrous or woody material and which imparts to such substances their property of rigidity. The process by which the cellulose is converted into ligno-cellulose is known as lignification. Just what this process involves in the way of chemical change and how it proceeds are still matters of dispute. Some investigators are of the opinion that it is purely a physical phenomenon; that the cellulose is simply embedded in, or incrusted by, the lignin; while others hold that the two are chemically combined and that the ligno-cellulose is formed at the expense of the cellulose.

Magnus (*30, p. 12*) considers that a definite linkage exists between the cellulose and the lignin.

Casparis (*7*) in a paper published in 1920 concludes that "lignified cell walls do not consist of chemically homogeneous material" and that "it appears likely that intramolecular formation of lignin takes place from the carbohydrates originally present in the cell wall."

Rassow and Zschenderlein (*34*) have evidence which points toward the pentosans as intermediate products in the formation of lignin.

Perhaps the most recent view of the process of lignification is that set forth by Esselen (*12*). He says in part:

> It has been demonstrated that lignin is made up of hydrosols of high molecular weight which are absorbed from the sap by the cellulose fibers. . . . The maximum lignification coincides with the maximum percentage of absorbable colloidal substances in the sap. While the lignification depends mainly on the absorption referred to, it may be followed by certain chemical reactions, particularly dehydration, which manifest themselves in toughening and ageing.

In whatever way the transformation is brought about, the final product is the highly complex ligno-cellulose, the ultimate structure of which still baffles the chemist. It is, however, generally agreed that the complex consists of cellulose linked in some way with two noncellulose substances, one of which contains an aromatic nucleus, while the other, be-

cause it yields furfural on distillation with HCl, is presumed to be pentosan in nature. The latter two substances are so closely associated that they are grouped together under the term "lignin," a complex containing a considerably higher percentage of carbon than does cellulose (about 60 per cent) and less resistant to the action of alkali. For this substance many formulae have been proposed. For a fairly complete list of these the reader is referred to a recent article by Beckmann, Liesche, and Lehmann (*4*). The most recent empirical formula possessed of any degree of definiteness is that of F. Lehmann (*4*), who suggests $C_{40}H_{44}O_{18}$. The work of Melander (*32*) is more recent, but his results are not conclusive, several formulae being suggested.

Klason's conception that lignin is allied to coniferyl alcohol and derived from it by condensation and oxidation is worthy of consideration.

The outstanding characteristic of lignin, and one on which almost all authorities agree, is the presence in the molecule of methoxy (CH_3O) and acetyl (CH_3CO) groups. These are readily split off by the action of heat and dilute alkali with the formation of acetic acid, the residue left behind being much more stable and insoluble than the original lignin.

There are data to show that some of the methoxy groups are contained in the cellulose, but this is not definitely established, and the generally accepted idea is that the methoxy is characteristic of lignin. The subject is well summed up by Schorger (*35*) in a treatise on the chemistry of wood.

THE ACTION OF SODIUM HYDROXID AND OTHER ALKALIES ON FIBER

When such material as straw or wood is macerated with a solution of an alkali the solution becomes colored dark brown, and if the process is continued sufficiently the material is more or less completely disintegrated into a pulpy mass, which can be bleached by chlorin or any other suitable bleaching agent to a white or nearly white substance. This residue is a crude form of cellulose, which, depending upon its subsequent use, may or may not be further purified.

What are the changes involved in the destruction of the complex lignified tissue? What is it that the alkali removes? From this point on we shall consider only the action of dilute alkali on straw, as our problem is not the production of cellulose but simply the utilization of the principle in sufficient degree to render the fiber more digestible, at the same time holding at a minimum losses of valuable food substances.

The most clear and concise explanation is that given by Magnus in his "Theorie und Praxis der Strohaufschliessung" (*30, p. 7–13*). He considers that the reaction proceeds in a threefold manner:

Separation and solution of the silicic acid, which constitutes a portion of the incrusting substance of the straw and is present in most straws to the extent of 1 to 2 per cent, while some straws contain as much as 5 per cent.

Splitting off of the methoxy and acetyl groups from the lignin, of which they form a characteristic part. This results in the production of acetic acid with consequent neutralization of more or less of the alkali employed in the process. The lignin itself is also profoundly changed and is rendered more insoluble and inactive. It should be borne in mind, however, that complete, or nearly complete, solution and removal of the lignin can be brought about when desired by repeated treatment with alkali at higher temperatures than those successfully employed for straw hydrolysis. In paper manufacture this is what actually takes place.

Forcing or springing of the bonds which link the lignin and cellulose together. The theory of a linkage between these two substances in the fiber is advanced by Magnus, and he considers that the springing apart of these bonds is the most important

and essential feature in the action of the alkali. As a result, the intestinal bacteria of animals are enabled to attack the cellulose and split it up into simpler substances, such as sugars and organic acids which can then be utilized by the animal organism.

These changes take place at ordinary temperatures and the maximum action of the alkali is reached in a comparatively short time. It should be said, in addition, that coincident with these favorable changes there is more or less destruction of pentosans and cellulose by the alkali, but in the improved process patented by Beckmann (*3*) this unfavorable action is held at a minimum.

Neger (*33*) has advanced the idea that the mechanical effect of the alkali on the straw is also important, the middle lamella of the cell wall being dissolved and the thick-walled cells separated from one another.

The action of calcium hydroxid is similar to that of sodium hydroxid, but is less marked, the lignin and silicic acid being less attacked.

DEVELOPMENT OF THE PROCESS OF HYDROLYSIS OF FIBER

An endeavor has been made to cover the subject matter on this particular phase of the problem as completely as possible.

Practically all the work of developing a suitable process of fiber hydrolysis has been carried on in Germany, straw being the material generally used. Although many of the investigations were a result of the acute food shortage in that country during the war, the idea of utilizing processed fiber as an animal or even a human food is by no means a new one.

As early as 1865 Hellriegel and Lucanus (*19*) investigated the feeding value of straw which had been chopped up, moistened, and allowed to heat spontaneously. They concluded that such treatment diminished somewhat the food value of the straw.

In 1890 Henneberg and Lehmann (*27*) carried on feeding experiments with crude fiber prepared from rye straw by the action of sodium hydroxid. They concluded that cellulose prepared in this way was nearly equal in value as an albuminoid conserver to the easily soluble carbohydrates, and also that cellulose aided in fat production.

In 1894 Lehmann, as reported by Kellner (*26, p. 288*), showed that the food value of straw could be increased by cooking it with caustic soda in ordinary open kettles. In 1902 (*28*) he modified his process and made use of the pressure cookers of the paper industry, heating the straw and soda lye under pressure for several hours. The digestibility of straw thus treated was raised from 42 per cent to 56 to 60 per cent. However, the process has not come into general use.

In 1899 Kellner (*26, p. 288*) observed that rye straw hydrolyzed by the process used in paper manufacture had a digestibility of 88 per cent and was capable of producing more fat in ruminants than pure potato starch.

In 1906 Ustiantzev (*44*) found that cellulose from straw freed from incrusting substances had a decided food value and was equal to isodynamic quantities of starch and sugar as a protector of protein and fat. When fed to both rabbits and sheep it was almost completely digested.

Altmannsberger (*1*) in 1907 fed sheep with straw that had been treated with sodium hydroxid under pressure and found that the straw was readily eaten and that the digestibility of the crude fiber and ash had been materially increased.

About the same time, Diffloth (*10*) published data showing the increased value as a feeding stuff of straw from which the incrusting substance had been removed.

Grégoire (*17*) reported in 1907 on the method of Seidl and Bauriedl in which straw was treated under pressure with 3 per cent NaOH. The material was fed while still wet and was claimed to be fully as digestible as starch.

After the outbreak of the World War, investigations of this nature became quite numerous. One of the first processes proposed was that of Oexmann, who utilized the straw pulp of the paper industry, mixing it with 35 per cent of molasses. The mixture was dried, ground, and placed on the market as "Strohkraftfutter II." With protein added to it, it was known as "Strohkraftfutter I."

Lehmann's process of cooking the straw under pressure with varying concentrations of NaOH was also further experimented with at this time, but was found to be not economically sound, due to costly equipment and the necessity of handling large amounts of water.

Colsmann's process was devised to overcome these defects and consisted of cooking straw without pressure for 12 hours, using simple equipment. The element of time was the main consideration here, and in that respect the Muller process, which shortened the time somewhat by stirring, was an improvement over Colsmann's method.

The Dahlemer process is similar in principle to Colsmann's and to Lehmann's original method, cast-iron vessels being employed for the hydrolysis of the straw.

Fingerling reports that Colsmann's product had a digestibility of 60 to 65 per cent (*26, p. 290*), while that of the Dahlemer process was 75 per cent digestible (*13, p. 6*).

Unfortunately, we have not been able to find in the literature any original accounts of the Oexmann, Colsmann, Muller, and Dahlemer processes, but they are reported in some detail by Fingerling (*26, p. 289, 290*) and by Magnus (*30, p. 1–4*). Because of the lack of references we are unable to assign definite dates as to the chronological sequence of their publication or introduction into practice. It is inferred, however, from a careful study of the literature that they were all developed during the early part of the war. Other investigators about the same time were Stutzer (*42*), Dannfelt (*9*), Tollens (*43*), and Hansen (*18*).

All of the processes devised for straw hydrolysis up to as late as 1917 required as an essential feature of their operation the application of heat. In 1918 Beckmann put forward his process of hydrolysis in the cold, which was so much more simple and economical that it rapidly superseded those already in use and became the subject of careful investigation by the German experiment stations and others. Since that time practically all the literature on the subject (in Germany at least) deals with this process or modifications of it. The process has been patented, in Germany about 1919, and in the United States more recently (*3*).

The essential features of the method are hydrolysis of the material with eight times its weight of 1.5 per cent NaOH in open vats for a comparatively short time—three hours is usually sufficient—draining off the liquor and washing with water until the product no longer turns red litmus paper blue. The process is carried on at ordinary temperature, and those who have thoroughly investigated the method claim that the hydrolysis is as complete at this temperature and in the relatively short time recommended as it is when the material is subjected to cooking either with or without pressure for longer periods of time. Also, the loss of valuable nutritive substance is very much reduced.

The method is discussed in considerable detail by Magnus (*30*) and also by Fingerling (*26, p. 291, 292*).

Magnus has reviewed and discussed in his text on the subject (*30*) all of the more important processes of straw hydrolysis which had been devised up to the date of its publication in 1919. In addition, he has given a detailed account of the theory of straw hydrolysis and of many of his own investigations. Though rather out of date now, it is the only text on the subject of which the writer is aware.

In 1919 Jonscher (*25*) reported on his investigations in treating straw meal and wood meal with HCl and (or) NaOH, recommending some of the products as suitable for animal and human food.

Within the past four years Honcamp and his coworkers have carried on quite extensive investigations into the relative merits of various methods of straw hydrolysis. In his first paper (*21*), published in 1919, he reports unfavorably on the methods proposed by Minck and Schwalbe for hydrolysis with hydrochloric acid. Such treatment makes the straw no more digestible.

His second paper (*22, p. 1–41*), published in 1921, deals with hydrolysis of straw by calcium hydroxid without pressure. Such treatment increases the starch value above that of the original straw to about the same extent as does sodium hydroxid. Loss of organic substance is greater when the hydrolysis takes place under pressure than by simply boiling.

In his third contribution (*22, p. 42–63*), published coincident with the second, he discusses the effect of hydrolyzing with sodium carbonate, which is similar to that of NaOH and Ca $(OH)_2$. Using concentrations of Na_2CO_3 similar to those of NaOH and $Ca(OH)_2$ employed, the fodder value of the straw was considerably improved.

His fourth paper (*23*), published about the same time as the second and third, deals with hydrolysis by sodium hydroxid under pressure. Only cereal straw is suitable for such treatment. Results obtained with a definite amount of NaOH (3.5 kg. per 100 kg. of straw) were about the same as where twice that amount was used.

In his most recent work (*24*) Honcamp investigated the Beckmann methods, using both sodium hydroxid and calcium hydroxid. His conclusion was that the loss in crude and digestible nutrients was greater with NaOH than with $Ca(OH)_2$.

In addition to this series of five papers, he published a general paper (*20*) in 1919 with some rather important conclusions. He states that pressure cooking results in greater destruction of organic matter than when the cooking is done in open vessels, and that hydrolysis with NaOH results in a substantial increase in digestibility in rye, barley, and oat straw, but only slight increase in pea, seed beet, and rape straw.

Semmler and Pringsheim (*40*) found that usually less than 50 per cent of the crude fiber of natural products is digested when the lignin content is in excess of 20 per cent, but up to 75 per cent may be digested in the case of straw hydrolyzed by sodium hydroxid, despite a much higher lignin content.

Fingerling has made some important contributions to the subject. In addition to his numerous experiments already referred to (*13, and 26, p. 289–292*), he has investigated Beckmann's process quite thoroughly. In 1919 he published a paper (*14*) dealing with the influence of time of hydrolysis upon the amount of nutritive material liberated. The results showed that the greatest amount of hydrolysis took place in the first three hours, and that action of the NaOH was practically completed

in four hours. In a second paper (*15*), published in 1922, he shows quite conclusively that, within reasonable limits, the stronger the NaOH solution used the higher is the digestibility of the hydrolyzed straw.

Wagner and Schöler (*46*) treated straw with 2 per cent lye by the Beckmann's process and found that the product when fed to sheep was very serviceable fodder, fed either wet or dry.

The work of Scurti et al., reported in 1919 and later, is worthy of mention. They have investigated the influence of hydrolysis on the composition and nutritive value of corncobs (*36*), wheat straw (*37*), grapevine shoots, and hemp (*38*). While sulphuric acid was the principal hydrolyzing agent used, nitric and hydrochloric acids and sodium hydroxid were also employed. The products from wheat straw and corncobs were compressed into cakes and fed to farm animals with fair success (*39*).

In 1919 Ellenberger (*11*) reported some experiments with hydrolyzed wood meal as a feed for working horses. He concludes that this material may not only be substituted for the hay of the ration but may also replace the oats if some supplemental protein is furnished.

Völtz (*45*) in 1920 treated straw and chaff by Beckmann's process, and reports considerably more digestible nutrients in chaff treated for 18 hours than in chaff treated for 3 hours. Straw treated for 24 hours contained slightly more digestible nutrients than straw treated for 12 or 72 hours.

In 1920 Godden (*16*) published an account of his method of straw hydrolysis, which he devised for small-scale operations and which differs somewhat from any of the German processes. The chopped straw is soaked overnight in 1.5 per cent of NaOH and then steamed for an hour in a specially constructed boiler. After draining and cooling it is fed immediately. The dry matter of the treated straw has approximately 1.5 times the value of the original dry matter, and for production purposes its value is nearly doubled. He concludes that the gain in nutritive efficiency compensates for the loss in dry matter, but emphasizes the need of further investigation of the possibilities of such treatment.

Weiser and Zaitschek (*47*) carried on an investigation similar to one by Fingerling (*15*) in which they studied the effect of the amount of soda used on the digestibility of straw. The Lehmann apparatus was used and, contrary to the findings of Fingerling, they found that the highest starch values were obtained when the NaOH solution used was weakest. From the large number of variables in their experiments we are inclined to view their results with some misgivings.

Sherrard and Blanco (*41*) have described a method for preparation of a cattle food from hydrolyzed sawdust. The product was fed to three cows at the Wisconsin College of Agriculture "with highly gratifying results." The essential feature of the method consisted in the digestion of the sawdust with 1.8 per cent sulphuric acid under pressure. About 21 per cent of the original wood meal was converted into sugar.

Braunschild (*6*) patented in 1921 a process for treatment of substances rich in cellulose with a strong solution of calcium chlorid.

Blasweiler (*5*) has recently described Steffen's method of straw digestion. Straw is cooked under pressure for one and one-half hours with 10 per cent sodium hydroxid. The product has a composition similar to that obtained by Oexmann's process.

From the many investigations cited, it is clear that the action of various hydrolyzing agents upon straw, while attended with some loss, noticeably improves its digestibility. Sodium hydrate proved to be the most effective agent, followed closely by calcium hydrate, the latter naturally proving the more economical.

EXPERIMENTAL

As already stated, the method of hydrolysis employed for the treatment of grain hulls in this investigation was that of Beckmann. The apparatus consisted of a tank constructed of 2-inch spruce planking, coated on the inside with asphalt, and provided with a strainer and outlet tap at the bottom to drain off the lye and wash water. The inside dimensions of this tank are: Length, 6 feet; breadth, 3 feet; depth, 1 foot 6 inches. Fifteen kilos of grain hulls can be readily handled in it at one time. In addition to the tank, a homemade filter press, for removal of the excess water after hydrolysis, and an eight-compartment special drying oven constituted the major portion of the equipment.

The procedure in treatment of the hulls was as follows: An amount of sodium hydroxid equivalent in weight to eight times the amount of hulls used was made ready in the tank, the exact strength being adjusted by titration and addition of more NaOH or water as required. The desired amount of hulls was weighed out and transferred at once to the tank, where it was thoroughly mixed with the alkali by means of a wooden hoe. The strengths of sodium hydroxid used were 1, 1.5, and 3 per cent. The 1.5 per cent strength is that employed by Beckmann. The 3 per cent strength, used with rice hulls only, was employed in order to ascertain whether it would have any more marked action on the very woody, gritty rice hulls than did the 1.5 per cent strength. The 1 per cent strength was used with the idea of economy in mind. The mixture was allowed to stand for three hours, with frequent stirring; the soda liquor was then drained off as completely as possible and the hulls thoroughly washed with cold water until the wash water no longer showed a pink tinge with phenolphthalein, about six changes of water being usually sufficient. The hulls were then transferred to the filter press, where the excess water was removed, and they were finally spread out in as thin layers as possible in shallow galvanized pans and dried in the special steam oven. When dry they were bagged and stored until such time as the digestion experiments could be carried on, which, as a matter of fact, was almost immediately.

As in all the digestion work done at this station, sheep were used, all the individuals employed being aged wethers well trained in the routine of the work. This is a detail of no small significance, as anyone can attest who has attempted work of this kind. The feeding trials were carried on in the usual manner, ample details of which are given in an earlier publication of this station (*29*). Two sheep were used throughout each trial. The hulls, both treated and untreated, were fed at the rate of 100 gm. [5] daily, along with a basal ration [6] of 500 gm. English hay, 150 gm. gluten feed, 10 gm. salt, and water ad libitum.

The hulls were mixed with the gluten feed, and no trouble was experienced in getting the sheep to eat them. No further preparation of the hulls was necessary except in the case of the rice hulls, which had to be ground before the sheep would eat them.

Feeds and feces were submitted to the regular fodder analyses, according to the methods of the Association of Official Agricultural Chemists, [7]

[5] In the case of the untreated oat hulls and rice hulls, and the oat hulls and rice hulls treated with 1.5 per cent sodium hydroxid, the amount of hulls fed was 150 gm.

[6] The basal ration for the trials in which untreated oat hulls and oat hulls treated with 1.5 per cent sodium hydroxid were fed contained no gluten feed: otherwise it was identical with that given above.

[7] Association of Official Agricultural Chemists. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS, AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. Revised to Nov. 1, 1919. *p. 71, 72, 97–98.* Washington, D. C. 1920.

and in addition the percentage of starch was determined in the hulls, and pentosans and lignin in all the feeds and solid excreta. Pentosans were determined according to the official method as described in the manual of the Association of Official Agricultural Chemists.[8]

Starch was determined by pancreatin in the following manner:

Two grams of finely ground (100-mesh) material were transferred to a hardened filter paper and washed with several portions of hot 10 per cent ethyl alcohol to remove the sugars. The residue was immediately transferred by means of a stemless funnel and a minimum of water from a wash bottle to a 250 cc. volumetric flask. The suspension, if not already diluted sufficiently by the water used for transferring, was further diluted to about 100 cc. and boiled for half an hour to rupture the cell walls and the starch granules and liberate the starch. The flask was then filled almost to the mark with distilled water and allowed to cool to below 37° C. A pinch of sodium bicarbonate was then added to insure slight alkalinity for optimum action of the pancreatin, followed by one-tenth of a gram of full-strength pancreatin.[9] The solution was at once made up to the mark, shaken well, and placed in a water bath at 37° to 40° C. for half an hour, at the end of which time the contents of the flask were emptied into a 500 cc. beaker to facilitate subsequent pipetting; 200 cc. of the liquid were immediately pipetted off into another 500 cc. beaker as rapidly as possible, and 20 cc. of HCl (sp. gr. 1.125) added at once, thus inactivating the pancreatin. The 220 cc. of liquid were then filtered by suction into another 250 cc. flask, using a platinum cone to support the filter paper, and a bell jar of suitable size on a ground glass plate to hold the flask into which the filtrate passed. As soon as possible after filtration was complete, the flask was placed under a reflux condenser and heated gently for two hours, the solution cooled, nearly neutralized with NaOH, and made up to 250 cc. With some materials another filtration was necessary at this point, but suction was not required. Reducing sugar was determined in aliquots of the solution by Allihn's modification of Fehling's method.[10]

Lignin was determined by a modification of the method of Ost and Wilkening (reported by Cross and Bevan, *8*, *p. 39*), which was proposed by Mahood and Cable (*31*). The principle of the method consists in the hydrolysis of all the constituents of the material except the lignin by means of concentrated sulphuric acid. The dissolved substances are removed by filtration and washing and the residue is dried and weighed as lignin. The details are as follows:

Two [11] grams of the material were extracted with ether in an ordinary fat extraction apparatus, transferred to a 1,000 cc. Erlenmeyer flask and covered with 10 times its weight (13 cc.) of 72 per cent sulphuric acid. Considerable care and some practice were necessary at this point in order to get all particles of the dry material in contact with the relatively small amount of acid. The hydrolysis was allowed to proceed for 16 hours at room temperature, at the end of which time the solution was diluted with ordinary tap water to a strength of 3 per cent (480 cc. of water was the amount necessary for that degree of dilution). The solution was then boiled under a reflux condenser for 2 hours, filtered through linen, washed with hot water, transferred to a tared Gooch crucible, dried at 100° C., weighed, ignited, and weighed again. The loss in weight was considered as lignin.

Some preliminary work was done in determining lignin by the method of Willstätter (reported by Magnus, *30*, *p. 14*), in which 42 per cent hydrochloric acid is used as the hydrolyzing agent, instead of sulphuric acid. This method proved less satisfactory because of the cost of HCl so highly concentrated, the difficulty of keeping it at that strength, and the extreme unpleasantness of the dense fumes given off.

[8] OP. CIT., *p. 96.*
[9] This material was furnished to us through the courtesy of Parke, Davis & Co., of Detroit, Mich., and, unlike the ordinary reagent, contained no diluent.
[10] Association of Official Agricultural Chemists. OP. CIT., *p. 90.*
[11] Mahood and Cable (*31*) use 4 gm., but half that quantity was better adapted to our purposes.

PRESENTATION AND DISCUSSION OF EXPERIMENTAL DATA

The results secured in the investigation group themselves under two heads: Effect of the sodium hydroxid on the composition of the hulls; effect on the digestibility and feeding value of the hulls.

EFFECT OF SODIUM HYDROXID ON THE COMPOSITION OF THE HULLS

This group of results can be subdivided into four distinct topics, which follow:

LOSSES IN WEIGHT IN THE TREATED MATERIALS DUE TO THE ACTION OF THE SODIUM HYDROXID, AND REDUCTION IN THE STRENGTH OF THE SODIUM HYDROXID

The loss in weight was ascertained on a dry-matter basis by weighing the material and making dry-matter determinations, both before and after treatment. The reduction in strength of the soda was determined by titration with N/2 sulphuric acid. The following table sets forth the figures:

TABLE I.—*Loss in weight on treatment with soda and reduction in strength of soda.*

Material.	Loss in weight on treatment (dry-matter basis).	Strength of NaOH employed.	Strength of NaOH after use.	NaOH consumed.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Oat hulls	16. 54	1. 5	0. 70	53. 33
Do	10. 74	1	. 46	54. 00
Barley hulls	20. 29	1. 5	1. 04	30. 66
Do	19. 31	1	. 64	36. 00
Rice hulls	15. 60	1. 5	. 75	50. 00
Do	19. 51	3	2. 28	24. 00
Cottonseed hulls	9. 69	1. 5	. 75	50. 00
Flax shives	25. 03	1. 5	. 80	46. 66

These losses are due, as already explained, to solution and separation of the silicic acid, a portion of the lignin, more or less of the cellulose and pentosans, and to unavoidable mechanical loss. In draining off the soda liquor and subsequent wash water, more or less of the fine particles of the treated hulls passed through the finest sieve that it was practicable to use. This was especially true of the flax shives, as they were ground quite fine when received. The mechanical loss, however, is believed to have been small.

The average loss in weight for the several substances when 1.5 per cent NaOH was used was equivalent to 17.43 per cent of the original material; when 1 per cent NaOH was used the average loss was slightly less—15.03 per cent; and in the single instance where 3 per cent NaOH was employed the loss was slightly higher—19.5 per cent; indicating that the stronger the soda solution the greater the loss, and that for a given strength of soda the loss depended upon the character of the material treated.

The reduction in strength of the sodium hydroxid is due to neutralization by the acetic acid formed. In this connection it may be worth while to record our observation of what took place when the hulls were added to the NaOH solution in the tank. During the first

few minutes of the treatment there was always a noticeable formation of small bubbles, accompanied by a quite audible crackling sound suggestive of a mild effervescence. This phenomenon ceased to be perceptible after the first 15 or 20 minutes, which would seem to indicate a slowing up of the reaction and that the action of the alkali takes place largely in the first few minutes. These assumptions are borne out by the work of Magnus (*30, p. 12*) and of Beckmann (*3*).

The average amount of alkali consumed or neutralized in the process was 45 per cent of the total amount when the strength employed was 1 per cent; 46.66 per cent when the strength employed was 1.5 per cent; 24 per cent when the strength employed was 3 per cent. In other words, the percentage strength of the exhausted solutions averaged 0.45 per cent, 0.70 per cent, and 2.28 per cent, respectively, for the 1 per cent, 1.5 per cent, and 3 per cent NaOH solutions.[12]

It will be noted that the percentage amount of NaOH consumed is about the same for the 1 per cent and 1.5 per cent—around 45 per cent, or slightly less than half of the total amount employed—while the actual amount consumed is about the same for the 1.5 per cent and 3 per cent solutions, namely, about 0.7 per cent.

Beckmann recommends the use of the exhausted alkali a second and even a third time, bringing it back to the desired strength by adding the required amount of fresh sodium hydroxid. In the practical operation of the process this procedure is in the interest of economy, but for our experimental work we considered it inadvisable, and accordingly a fresh solution was prepared for each lot of hulls.

REACTION OF THE MATERIALS BEFORE AND AFTER TREATMENT

In order to ascertain if there was any residual uncombined alkali in the treated materials, the water-soluble acidity or alkalinity of all the treated and untreated samples was determined according to the method given in the manual of the Association of Official Agricultural Chemists.[13] Table II sets forth the results.

TABLE II.—*Water-soluable acidity or alkalinity of untreated and treated hulls*

Laboratory No.	Material.	N/10 NaOH required per gram of substance.	N/10 H_2SO_4 required per gram of substance.
		Cc.	*Cc.*
248	Oat hulls, untreated	0.23	
503	Oat hulls, treated 1 per cent NaOH	.10	
293	Oat hulls, treated 1.5 per cent NaOH		0.16
453	Barley hulls, untreated	1.14	
491	Barley hulls, treated 1 per cent NaOH	.23	
482	Barley hulls, treated 1.5 per cent NaOH	.42	
337	Rice hulls, untreated	.22	
321	Rice hulls, treated 1.5 per cent NaOH		.07
512	Rice hulls, treated 3 per cent NaOH		.08
449	Cottonseed hulls, untreated	.23	
460	Cottonseed hulls, treated 1.5 per cent NaOH	.08	
485	Flax shives, untreated	.47	
494	Flax shives, treated 1.5 per cent NaOH	.08	

[12] The 3 per cent NaOH solution was used in only one instance, hence the corresponding value does not represent an average.

[13] Association of Official Agricultural Chemists. OP. CIT., *p. 98*.

It is seen that all untreated materials showed a slight water-soluble acidity measured in terms of N/10 NaOH, varying from 0.22 cc. N/10 NaOH in the case of rice hulls to 1.14 cc. in the case of barley hulls. A slight acidity is normal to most feeding stuffs. The relatively high acidity of the barley hulls may be attributed to a slight fermentation of the considerable amount of carbohydrate present which had not been separated from the hulls. In the case of the treated materials, five lots showed a slight acidity, varying from 0.08 cc. to 0.42 cc. N/10 NaOH, and three lots showed a slight alkalinity, from 0.07 cc. to 0.16 cc. N/10 H_2SO_4 per gram of substance. From these results it is evident that in the majority of cases there was no residual uncombined soda from the treatment left in the materials after thorough washing, and in those cases where there was any alkalinity it was so slight that it would be readily taken care of by the HCl in the animal's stomach. Care should, however, be used to wash out the soda after treatment as thoroughly as possible compatible with good practice.

EFFECT OF THE TREATMENT ON THE PROXIMATE CONSTITUENTS OF THE HULLS

The detailed results of all the proximate analyses, together with percentage increases or decreases of the various constituents due to the action of the alkali, are given in the accompanying table.

TABLE III.—*Summary of the proximate analysis of grain hulls (untreated and treated)*

Laboratory No.	Material.	Moisture as fed.	Dry matter basis.				
			Total ash.	Crude protein.	Crude fiber.	N-free extract.	Crude fat.
248	Oat hulls, untreated	7.70	6.33	2.26	33.24	57.24	0.93
503	Oat hulls, treated 1 per cent NaOH	5.77	5.69	2.05	34.80	56.99	.47
	Percentage increase or decrease due to treatment		−10.12	−9.30	+4.69	−.44	−49.47
293	Oat hulls, treated 1.5 per cent NaOH	4.05	5.23	1.37	40.20	52.65	.55
	Percentage increase or decrease due to treatment		−17.38	−39.39	+20.93	−8.02	−40.86
337	Rice hulls, untreated	6.25	19.06	3.02	41.80	35.38	.75
321	Rice hulls, treated 1.5 per cent NaOH	3.61	17.46	2.31	46.08	33.41	.58
	Percentage increase or decrease due to treatment		−8.40	−16.89	+10.23	−5.57	−22.67
512	Rice hulls, treated 3 per cent NaOH	5.92	13.13	1.83	50.26	34.23	.55
	Percentage increase or decrease due to treatment		−31.12	−39.40	+20.23	−3.25	−26.67
453	Barley hulls, untreated	6.76	4.50	10.81	15.56	66.86	2.27
491	Barley hulls, treated 1 per cent NaOH	6.24	4.74	10.41	18.97	63.89	1.99
	Percentage increase or decrease due to treatment		+5.33	−3.63	+21.91	−4.45	−12.34
482	Barley hulls, treated 1.5 per cent NaOH	5.09	4.33	9.25	19.03	65.40	1.99
	Percentage increase or decrease due to treatment		−3.78	−14.44	+22.30	−2.18	−12.34
449	Cottonseed hulls, untreated	6.93	2.13	4.08	43.99	48.60	1.19
460	Cottonseed hulls, treated 1.5 per cent NaOH	5.15	2.75	3.03	49.83	43.55	.84
	Percentage increase or decrease due to treatment		+29.10	−25.74	+13.27	−10.40	−29.42
485	Flax shives, untreated	6.78	4.09	5.24	53.81	35.05	1.81
494	Flax shives, treated 1.5 per cent NaOH	5.54	5.35	4.41	62.84	26.07	1.33
	Percentage increase or decrease due to treatment		+30.81	−15.84	+16.77	−25.63	−26.52

\+ Increase. − Decrease.

The treatment relatively increased the fiber and decreased all other constituents except ash, which was increased in three instances. The considerable increase in ash in cottonseed hulls and flax shives can probably be explained by considering it as a relative increase, the ash of these

materials being so insoluble that it was unattacked by the alkali, while a portion of the more soluble organic constituents was removed. It is possible also that sufficient residual soda remained combined in these materials to account for the ash increase.

The striking feature of the results is the appreciable percentage increase of fiber in all cases, due to the removal of the more soluble portions of the hulls. One notices also that the increase in fiber was consistently greater the higher the concentration of sodium hydroxid. Although the protein and fat suffered considerable loss, they are present in relatively small percentages and hence are of minor importance. (See Table IV.)

TABLE IV.—*Net loss in pounds on a dry-matter basis of each proximate constituent*

Material.	Loss in pounds for each 100 pounds of dry matter treated.					
	Total ash.	Crude protein.	Crude fiber.	N-free extract.	Crude fat.	Total.
Oat hulls, treated 1 per cent NaOH	1.26	0.43	2.18	6.37	0.51	10.75
Oat hulls, treated 1.5 per cent NaOH	1.97	1.12	[a].31	13.30	.47	16.55
Barley hulls, treated 1 per cent NaOH	.68	2.41	.25	15.31	.66	19.31
Barley hulls, treated 1.5 per cent NaOH	1.05	3.44	.39	14.73	.68	20.29
Rice hulls, treated 1.5 per cent NaOH	4.32	.90	2.91	7.18	.26	15.57
Rice hulls, treated 3 per cent NaOH	8.49	1.55	1.35	7.83	.31	19.53
Cottonseed hulls, treated 1.5 per cent NaOH	[a].35	1.34	[a]1.01	9.27	.43	9.68
Flax shives, treated 1.5 per cent NaOH	.68	1.93	6.70	15.51	.81	25.03

[a] These figures represent gain instead of loss. In the case of the crude fiber an absolute gain is impossible, so these small increases are explainable only on the basis of analytical or experimental error. In the case of the ash which showed an absolute increase it is possible that residual NaOH might account for it.

Considering the results from this angle, we see that the fiber was practically unattacked in so far as its removal by solution was concerned, while the greater or lesser amounts of all the other constituents were removed. The greatest actual losses were in the case of the nitrogen-free extract, which includes the starch, a portion of the pentosans and lignin, and allied substances.

EFFECT OF THE TREATMENT ON SOME OF THE ULTIMATE CONSTITUENTS OF THE HULLS

It was thought that a more detailed analytical examination of the hulls than that involved in the conventional fodder analyses would furnish still more accurate information as to the chemistry involved in the process. Table V gives the results of starch, pentosan, and lignin determinations made as described in a previous section of this paper.

The figures in Table V show that relatively the starch was increased in all instances, the pentosans in all but one instance, and the lignin in five out of eight. In a general way, the increases in pentosans and lignin parallel those for fiber, which is what would be expected.

One is impressed with the high percentage of pentosans in the untreated oat hulls; in fact, pentosans together with the lignin make up the major portion of the hulls. The rice hulls are composed largely of ash, fiber, including pentosans, and lignin. They are somewhat more lignified than the oat hulls, and the presence of the lignin together with the high ash percentage accounts for their inferior nutritive value. Cottonseed hulls with their very high crude fiber percentage, together with the large amount of pentosans and lignin, are in the same class with the rice hulls. Flax shives, containing approximately 54 per cent

of fiber, 27 per cent of pentosans, and 33 per cent of lignin, the most lignified of the several substances examined, should prove to be the least digestible.

TABLE V.—*Starch, pentosans, and lignin in grain hulls (untreated and treated)*

Material (dry matter basis).	Starch per cent.	Pentosans per cent.	Lignin per cent.
Oat hulls, untreated	4.73	40.02	20.20
Oat hulls, treated 1 per cent NaOH	5.59	43.80	23.54
Percentage increase or decrease due to treatment	+18.18	+ 9.45	+16.53
Oat hulls, treated 1.5 per cent NaOH	7.67	44.16	18.61
Percentage increase or decrease due to treatment	+62.16	+10.34	− 7.87
Rice hulls, untreated	5.65	21.98	22.72
Rice hulls, treated 1.5 per cent NaOH	5.67	24.10	23.09
Percentage increase or decrease due to treatment	+ 0.35	+ 9.65	+ 1.63
Rice hulls, treated 3 per cent NaOH	5.67	25.28	25.10
Percentage increase or decrease due to treatment	+ 0.35	+15.01	+10.48
Barley hulls, untreated	13.41	23.50	14.87
Barley hulls, treated 1 per cent NaOH	15.78	26.61	15.83
Percentage increase or decrease due to treatment	+17.67	+13.23	+ 6.46
Barley hulls, treated 1.5 per cent NaOH	22.55	26.21	15.01
Percentage increase or decrease due to treatment	+68.16	+11.53	+ 0.94
Cottonseed hulls, untreated	5.87	32.52	25.29
Cottonseed hulls, treated 1.5 per cent NaOH	6.08	34.10	21.97
Percentage increase or decrease due to treatment	+ 3.58	+ 4.86	−13.13
Flax shives, untreated	6.26	27.16	33.28
Flax shives, treated 1.5 per cent NaOH	6.89	25.92	32.40
Percentage increase or decrease due to treatment	+10.06	− 4.57	− 2.64

\+ Increase. − Decrease.

The losses due to treatment seem to be distributed between the ash, protein, fat, pentosans, and lignin. The most pronounced losses occur in the pentosans and lignin. Even after all of these are accounted for, there is more or less loss of alkali-soluble constituents not identified, which because of a lack of complete identification are still grouped under the term nitrogen-free extract. In fact, in substances of this nature the nonnitrogenous materials are of such a complex nature and are so interwoven with each other that it does not appear possible to determine the different constituents with quantitative exactness. This difficulty in the case of starch has led Armsby (*2, p. 72*) to remark that "unfortunately starch can be determined only more or less approximately."

TABLE VI.—*Net loss of starch, etc., due to treatment*

Material.	Loss in pounds for each 100 pounds of dry matter treated.		
	Starch.	Pentosans.	Lignin.
Oat hulls, treated 1 per cent NaOH	[a] 0.26	0.98	[a] 0.81
Oat hulls, treated 1.5 per cent NaOH	[a] 1.67	3.16	4.67
Barley hulls, treated 1 per cent NaOH	.68	2.03	2.10
Barley hulls, treated 1.5 per cent NaOH	[a] 4.56	2.61	2.91
Rice hulls, treated 1.5 per cent NaOH	.86	1.64	3.23
Rice hulls, treated 3 per cent NaOH	.91	1.63	2.32
Cottonseed hulls, treated 1.5 per cent NaOH	.38	1.72	5.45
Flax shives, treated 1.5 per cent NaOH	1.09	7.73	8.99

[a] Represents a gain, which of course is impossible and is explainable only on the basis of analytical or experimental error.

EFFECT OF SODIUM HYDROXID ON THE DIGESTIBILITY AND FEEDING VALUE OF THE HULLS

This is revealed by a study of the digestion coefficients of the various constituents of the hulls. For the purposes of this investigation we need consider only the digestion coefficients of the total dry matter, crude fiber, nitrogen-free extract, pentosans, and lignin. These are the important constituents, the protein, fat, and ash being present for the most part in unimportant amounts. Table VII presents the results in condensed form. For the benefit of the critical student it is appropriate to remark that the digestion trials were conducted with the greatest care. Where the coefficients from two individuals differ materially, it should be borne in mind that work of this nature can not be controlled in the same way that laboratory determinations can be. Biological processes are too complex to permit it; and it frequently happens also that the individuality of the animal exerts an influence on the final result. Accordingly the coefficients must be viewed as giving general rather than absolutely definite results.

Careful scrutiny of the individual and average digestion coefficients reveals the following facts:

The digestibility of the total dry matter was substantially increased by treatment in case of the oat, barley, and rice hulls.

The digestibility of the crude fiber, nitrogen-free extract, and pentosans in oat, barley, and rice hulls was markedly increased by treatment.

The results in case of lignin are not so consistent, but it must be remembered that treatment has changed its molecular structure, hence the data can not be considered of any particular value. It seems evident that in the untreated material, in several instances, some little use was made of the lignin complex, the results varying with the different materials. After treatment, in case of the oat, rice, and barley hulls the digestibility of the lignin residue seems to have been somewhat improved. In view, however, of our incomplete knowledge of the structure of the lignin molecule and of the varying results secured with two sheep on the same material, it may be concluded that lignin is of quite doubtful value as a source of nutrition. Some investigators consider it to be entirely indigestible.

In addition to the facts mentioned under Table VII, the following general statements seem to be warranted:

Expressed on a percentage basis, treatment with varying strengths of dilute NaOH invariably increased the total digestible dry matter of oat hulls, barley hulls and rice hulls. In the majority of cases it also increased markedly the digestibility of the important constituents of these materials.

Cottonseed hulls and flax shives appear to have been unaffected by the treatment. It is probable that the lignin-cellulose linkage was broken only to a slight extent, due no doubt to the higher degree of lignification in these materials, as compared with the hulls of the cereal grains.

Although the rice hulls showed by far the greatest response to the action of the soda, the original material was so much inferior in digestibility to the untreated oat and barley hulls that the net result of treatment was a product considerably inferior in total digestible nutrients even to the untreated oat hulls; hence the action of soda on this material is not likely to be of economic value.

TABLE VII.—*Digestion coefficients (individual results and average of two sheep)*

Material	Sheep.	Total dry matter.	Crude fiber.	N-free extract	Pentosans.	Lignin.
Oat hulls, untreated	15	36.37	56.32	31.73	33.43	11.23
Do	11	36.11	48.82	36.42	38.38	(a)
Average		36.24	52.57	34.08	35.91	
Oat hulls, treated 1 per cent NaOH	16	26.50	57.88	26.65	51.18	(a)
Do	17	70.37	82.77	64.67	71.07	39.63
Oat hulls, treated 1.5 per cent NaOH	12	73.31	84.91	71.55	84.56	28.15
Do	13	87.90	97.22	86.53	62.99	46.56
Average		80.61	91.07	79.04	73.78	37.36
Increase due to treatment (1 per cent NaOH)		[b] 34.13	[b] 30.20	[b] 30.59	[b] 35.16	[b] 28.40
Percentage increase		[b] 94.17	[b] 57.43	[b] 89.76	[b] 97.91	[b] 252.89
Increase due to treatment (1.5 per cent NaOH)		44.37	38.50	44.96	37.87	26.13
Percentage increase		122.43	73.24	131.92	105.46	232.68
Barley hulls, untreated	16	65.77	42.73	64.24	50.02	18.76
Do	17	53.17	46.86	55.47	44.77	(a)
Average		59.97	44.79	59.85	47.40	
Barley hulls, treated 1 per cent NaOH	16	64.56	37.99	70.04	52.87	23.85
Do	17	72.83	66.55	79.57	76.91	45.55
Average		68.69	52.28	74.81	64.89	34.70
Barley hulls, treated 1.5 per cent NaOH	16	79.49	70.76	81.98	73.91	(a)
Do	17	85.52	91.80	87.22	92.16	(a)
Average		82.50	81.28	84.60	83.04	
Increase due to treatment (1 per cent NaOH)		8.72	7.49	14.96	17.49	15.96
Percentage increase		14.54	16.72	25.00	36.90	84.97
Increase due to treatment (1.5 per cent NaOH)		22.53	36.49	24.75	25.04	(a)
Percentage increase		37.57	81.47	41.35	75.19	(a)
Rice hulls, untreated	9	(a)	(a)	14.57	3.34	(a)
Do	12	4.97	12.08	5.21	(a)	(a)
Average				9.89		
Rice hulls, treated 1.5 per cent NaOH	9	23.72	20.28	35.04	51.36	13.87
Do	11	34.60	36.67	41.04	60.43	18.06
Average		29.16	28.48	38.04	55.92	15.97
Rice hulls, treated 3 per cent NaOH	18	34.02	28.42	50.53	48.91	10.97
Do	19	33.96	22.71	39.57	29.27	21.73
Average		33.99	25.57	45.05	39.09	16.35
Increase due to treatment (1.5 per cent NaOH)		24.19	16.40	28.15	52.58	15.97
Percentage increase		486.72	135.76	284.63	1,674.25	[c]
Increase due to treatment (3 per cent NaOH)		29.02	13.49	35.16	35.75	16.35
Percentage increase		583.90	111.67	355.51	1,170.36	[c]
Cottonseed hulls, untreated	18	46.06	54.00	54.74	92.26	(a)
Do	19	59.98	62.41	62.66	88.10	31.26
Average		53.02	58.21	58.70	90.18	
Cottonseed hulls, treated 1.5 per cent NaOH	18	51.84	51.29	66.84	108.87	(a)
Do	19	57.21	53.83	68.53	92.70	9.45
Average		54.52	52.56	67.69	100.78	
Increase due to treatment		1.50	[d] 5.65	8.99	10.60	[d] 21.81
Percentage increase		2.83	[d] 9.71	15.32	11.75	[d] 69.77
Flax shives, untreated	18	(a)	(a)	[e] 3.92	[e] 6.91	(a)
Do	19	30.15	18.67	33.18	25.36	37.79
Flax shives, treated 1.5 per cent NaOH	18	18.31	20.15	31.34	39.09	(a)
Do	19	39.21	25.37	45.43	46.32	20.32
Average		28.76	22.76	38.38	42.71	
Increase due to treatment		[d] 1.41	4.09	5.20	17.35	[d] 7.47
Percentage increase		[d] 4.61	21.91	15.67	68.41	[d] 26.88

[a] Negative.

[b] For some reason sheep 16 did not digest the oat hulls fed in this trial at all well. As it is not wise to average results which vary so widely as do those for these two sheep on this material, the figures for sheep 16 in this trial are not made use of in subsequent calculations.

[c] Can not be computed; no basis to start from, as digestibility of lignin in the untreated material was nil.

[d] Decrease.

[e] It would be unwise to average these results. The figures for sheep 19 are employed in the subsequent calculations.

Where varying strengths of NaOH were used on the same material, an increase in strength of solution was almost invariably accompanied by a considerable increase in digestibility of the hulls, most marked when the comparison was between 1 per cent NaOH and 1.5 per cent NaOH, not so marked where 3 per cent NaOH was used. In this connection it should be noted that losses in weight due to treatment were slightly increased by an increase in strength of the soda.

TABLE VIII.—*Effect of varying strengths of sodium hydxoid in increasing digestibility of fibrous material* [a]

Solution used.	Percentage increase over untreated hulls.				
	Total dry matter.	Crude fiber.	N-free extract.	Pento-sans.	Lignin.
1 per cent NaOH (oats)	94.17	57.45	89.76	97.91	252.89
1 per cent NaOH (barley)	14.54	16.72	25.00	36.90	84.97
1.5 per cent NaOH (oats)	122.43	73.24	131.92	105.46	232.68
1.5 per cent NaOH (barley)	37.57	81.47	41.35	75.19	(b)
1.5 per cent NaOH (rice)	486.72	135.76	284.63	1,674.25	(c)
3 per cent NaOH (rice)	583.90	111.67	355.51	1,170.36	(c)

[a] The cottonseed hulls and flax shives are not included in this summary because of the negligible effect the soda solution had on their digestibility.
[b] Negative.
[c] Averages not available for lignin because of varying results in its digestibility.

As a rule, increases in the relative amount of a component due to treatment were accompanied by an increase in its percentage digestibility.

Taking into consideration both the loss in weight and the increased digestibility due to the action of the soda (see Tables IV and VI) we obtain net gains as shown in Table IX.

TABLE IX.—*Average net gain in total digestible dry matter and in the important digestible nutrients of oat hulls and barley hulls on the basis of 100 pounds of dry matter treated*

Material.	Total digestible dry matter.	Digestible crude fiber.	Digestible N-free extract.	Digestible pento-sans.	Digestible lignin.
Oat hulls, treated 1 per cent NaOH	7.72	5.47	3.60	11.81	[a] 7.06
Oat hulls, treated 1.5 per cent NaOH	31.04	[a] 19.14	11.59	13.88	2.94
Barley hulls, treated 1 per cent NaOH	[b] 4.55	2.81	[b] 3.68	4.81	1.97
Barley hulls, treated 1.5 per cent NaOH	5.79	8.18	11.31	8.46	(c)

[a] Referring to Tables IV and VI it will be seen that the total crude fiber and total lignin of oat hulls each showed a net gain instead of a net loss after treatment. As already noted, this is impossible, so the results in question are obtained by assuming that no loss of these constituents was involved.
[b] This result represents a net loss.
[c] Digestibility of lignin in this instance was a minus quantity.

Considering the net gains as shown in the table, it can be said that with one exception (barley hulls, 1 per cent NaOH) the increase in digestibility outweighed the loss by solution in the soda. It is questionable, however, whether in those materials showing the smaller gains the increase would offset the cost of treatment. The extra 0.5 per cent of sodium hydroxid apparently makes a great difference in the final result.

The barley hulls probably would have made a more favorable comparison with the oat hulls had they not contained so much starchy material. Unfortunately, the separation of the endosperm from the hull had not been nearly so complete as in the case of the oat hulls (due in part, probably, to the greater tenacity with which the barley hull clings to the endosperm, or possibly to a less perfect mechanical method of separation), and as a result there was quite a large amount of the floury portion of the grain adherent to the hulls.

On a dry-matter basis the digestible matter in a ton of oat hulls was increased by treatment with 1.5 per cent NaOH from 725 pounds to 1,345

pounds, the digestible crude fiber from 349 pounds to 732 pounds, and the digestible nitrogen-free extract from 390 pounds to 622 pounds. The digestibility of the pentosans, which are distributed between the fiber and nitrogen-free extract, was increased from 287 pounds per ton to 605 pounds. In short, the feeding value of the oat hulls was about doubled. It seems that such a result should warrant further investigation with the idea of making the process of economic importance.

SUMMARY

This paper reports the results of an investigation on the problem of increasing the digestibility and feeding value of grain hulls.

A review of the literature shows:

1. That the important constituent of the cereal and other straws is lignocellulose, a compound the chemistry of which is not fully understood. It is known, however, that it consists of cellulose linked in some manner with lignin and that the presence of the latter compound is characterized by the splitting off of methoxy (CH_3O) groups upon hydrolysis.
2. That the action of dilute alkali on fiber is threefold, consisting of separation of the silicic acid which forms a part of the incrusting material, splitting off of the methoxy groups of the lignin with formation of acetic acid, and springing of the bonds which exist between the lignin and cellulose.
3. That practically all of the work on this problem has been carried on in Germany, most of it since the commencement of the World War, and the material most generally investigated has been straw, the digestibility of which has been decidedly increased. Grain hulls do not appear to have been worked with heretofore.

In our investigation the method used for treatment of the hulls was that of Beckmann. The materials treated were oat hulls, barley hulls, rice hulls, cottonseed hulls, and flax shives. The hydrolyzing agent was cold dilute sodium hydroxid, the strengths employed being 1, 1.5, and 3 per cent.

Most satisfactory results were obtained with NaOH of 1.5 per cent strength; 1 per cent was apparently too dilute, and 3 per cent in the one instance used did not have sufficient increased action over the 1.5 per cent to warrant its use.

The effect of the alkali on the composition of the hulls was ascertained by the usual methods of fodder analysis, supplemented by determination of some of the ultimate constituents of the hulls both before and after treatment.

The result of treatment was a decrease in all proximate constituents except the crude fiber, which from an absolute standpoint remained practically the same but relatively was considerably increased.

Losses in weight due to the treatment were noticed in all materials; they were greatest in the case of the flax shives (25 per cent), and least in the case of the cottonseed hulls (9.7 per cent).

The effect of the alkali on the digestibility of the hulls was ascertained by the usual methods employed in digestion experiments, sheep being the animals used. As a result of the treatment with 1.5 per cent NaOH the digestibility of the important constituents of oat hulls and barley hulls was markedly increased, the feeding value of the oat hulls being doubled. The digestibility of rice hulls was also improved greatly, but not sufficiently to be of economic significance. The results with cottonseed hulls and flax shives were in the main negative.

Since the digestibility of oat and barley hulls is greatly improved by the action of dilute alkali, some method should be devised that could be applied on an economic scale. Also, a method for the improvement of the digestibility of cottonseed hulls is worthy of further attention.

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THE TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTONS AND THEIR F_1 HYBRID[1]

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INTRODUCTION

This paper has a twofold purpose: (*a*) The presentation of the results of an investigation of the physicochemical properties of the leaf tissue fluids of Egyptian and Upland cotton as grown under irrigation at Sacaton, Ariz.; (*b*) a comparison of the properties of the leaf tissue fluids of the F_1 hybrid between these two cottons with those of the two parent types.

The consideration of both of these groups of problems rests on the results of much antecedent work.

In a series of investigations carried out in natural plant habitats the writers have shown that there is a close relationship between the aridity of the habitat and the osmotic concentration of the plant tissue fluids of the native vegetation. These results, obtained by careful cryoscopic measurements in the southwestern deserts (*30*)[2], in the mesophytic habitats of the Eastern United States (*29*), in the Jamaican deserts (*26*) and rain forest (*28*), in the mangrove swamp (*27*), and in many other localities for which the data are not yet published, fully substantiate and greatly extend the general conclusions drawn from the earlier plasmolytic determinations by Drabble and Drabble (*14*) and by Fitting (*15*). That the osmotic concentration of the plant tissue fluids is a factor of importance in determining the capacity of the plant for survival under conditions of aridity is suggested by work on the relationship of the loranthaceous parasite to its host, as investigated by plasmolytic studies by Senn (*48*), and by cryoscopic studies on both rain forest and desert (*20, 25, 33*).

Concurrently with these investigations on the osmotic concentration and electrical conductivity of plant tissue fluids, another group of workers, among whom Wherry has been the most active, has furthered investigations on the relationship between soil acidity and plant distribution. In this place it will suffice to refer to the more recent reviews by Wherry (*51*) and by Atkins (*2*).

From the earliest stages of the investigations we realized the desirability of investigations of the sap properties of agricultural plants. Since natural vegetations have been shown to differ in the physicochemical properties of their tissue fluids, it would seem quite reasonable to assume

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[2] Reference is made by numbers (italic) to "Literature cited," p. 325-327.

that the properties of the tissue fluids of crop plants may be of importance in determining the possibility of their growth and productiveness under the varying soil and atmospheric conditions of different regions.

This assumption (which receives additional support from the work which has been done on native vegetation as an indicator of the suitability of land for crop production, as developed by Shantz (*49*), by Kearney, Briggs, Shantz, McLane, and Piemeisel (*41*), and by Clements (*11*) seems so reasonable that it becomes of the greatest importance to determine to what extent species and varieties which are of agricultural and horticultural importance are differentiated with respect to the physicochemical properties of their tissue fluids, and to ascertain in how far observed differences in these properties represent innate and relatively constant differences and to what degree they are the resultant of varying environment conditions, such as dryness and salinity of soil, insolation, and the evaporating power of the air.

From the beginning of these investigations[3] we also foresaw the possibilities of a consideration of the relation of the physicochemical properties of the plant tissue fluids to the relative vigor of hybrid and parental forms and to other problems in genetics. For the past several years we have, therefore, been seeking the materials and opportunities for an investigation of the physicochemical properties of the tissue fluids of heterozygous individuals as compared with those which are homozygous for one or many characters. It seemed desirable if possible to obtain parent forms which themselves differed in respect to such tissue-fluid properties as osmotic concentration, specific electrical conductivity, and hydrogen-ion concentration.

Cotton as grown in Arizona and California is of interest to the physiologist because it is of necessity grown on irrigated land where the salt content of the soil may be relatively high and because it is a plant with heavy and watery foliage, which must nevertheless be grown under the conditions of temperature and insolation of a fairly severe desert climate. Both of these factors—economic importance and peculiar conditions of growth—combine to render of special importance the investigation of any physiological peculiarities of the plant which may influence its capacity for growth under the rigorous conditions of the Southwest.

HISTORICAL

The earlier literature of Egyptian cotton as grown in Egypt has been summarized, or at least cited, by Balls (*4*), who has more recently considered certain problems of growth and yield (*7*). We make no attempt in this place to review the purely agronomic literature on American cultivation of Egyptian cotton, much of which is cited in a paper describing the American-Egyptian cotton industry, by Scofield, Kearney, Brand, Cook, and Swingle (*47*). Mention should be made also of the recent investigations of King (*43*) on water-stress behavior of Pima

[3] In 1910, Dr. T. H. Kearney, physiologist in charge of alkali and drought-resistant plant investigations, Bureau of Plant Industry, and Mr. G. N. Collins, botanist in charge of biophysical investigations, Bureau of Plant Industry, recognizing the possible agricultural bearing of the results already obtained from the studies of native vegetation, invited us to undertake investigations on the physicochemical properties of native indicator plants and of crop plants in the arid West.

These field operations have included studies on the native vegetation of Tooele Valley, Utah, of cereals grown under dry farm agriculture at Nephi, Utah, and on Egyptian and Upland cotton grown under irrigation at Sacaton, Ariz. While all these studies in the arid West are mutually supplementary, and should be considered in their entirety, they must be presented in sections. The present paper deals exclusively with a comparison of Egyptian and Upland cotton and their F_1 hybrid.

Egyptian cotton in Arizona and of the investigation of cross- and self-fertilization as related to the maintenance of purity of strains by Kearney (*39*). These furnish a key to the more important literature. The contents of a number of chemical papers on the cotton plant do not interest us in this connection.

Limiting our attention to the very meagre literature of the relationship of the cotton plant to soil salinity and dryness, we may note that 20 years ago Kearney and Means (*42*) found that in fields in the neighborhood of Alexandria, Egypt, where the washing used in reclamation had not yet been completed, the salt content of the upper 2 feet of the soil was, among good plants, 0.6 per cent; near occasional plants in partly bare ground, 1.8 per cent; on wholly bare ground, 2 per cent and higher.

"These figures indicate an exceptionally high degree of resistance in the cotton plant, marking it as one of the very foremost in this respect of the world's great crops." Further nonquantitative notes on the occurrence of cotton on saline land are given.

Finally, Kearney (*36*) has discussed the growth of Egyptian cotton on alkali soils. His results indicate that with alkali of the type found at Sacaton the fruitfulness of the plants is likely to be impaired when the salt content exceeds 0.4 per cent of the dry weight of the soil. This, he concludes, would seem to be about the limit for profitable production of this crop in the presence of alkali of the type found at Sacaton, although he observed that the quality of the fiber does not necessarily suffer in the presence of 0.55 per cent of salt. He found that the moisture capacity of the soil is an important factor in determining the size, vigor, and fruitfulness of Egyptian cotton plants. The alkali resistance of Egyptian cotton is relatively high when other conditions are favorable. It would appear that a fair yield of fiber of good commercial quality can be obtained when nearly one-half of 1 per cent of the total dry weight of the soil consists of readily soluble alkali salts, provided that carbonates are absent or form only an inconsiderable proportion of the total mineral solutes.

The only literature on the salt content of the Egyptian cotton plant of which we are aware consists of two short papers by Balls (*5*, *6*), both of which came to our attention after this manuscript was practically completed.

In the first of these (*5*) he calls attention to a chance result which indicates that the tree cottons and Egyptian cotton bring up from deeper layers and deposit on the surface of the soil through the shedding of their leaves sufficient quantities of substances, of which NaCl is one constituent, to render the soil unsuitable for other plantings.

In the second (*6*) he compares the chlorid content of three pure strains of Egyptian cotton grown at Gizeh, Egypt, on land which contained not more than 0.1 per cent of NaCl in the surface layers, even after long deprivation of water. From these analyses, which are few in number and based on the dry weight of the leaves only, he concludes that Egyptian cotton growing under typical field crop conditions has a salt content which indicates a concentration of 0.3 per cent NaCl in the leaf cell sap. This concentration varies with the salinity of the soil, though not proportionally. It also varies with the particular pure strain or variety. Plants of two Egyptian strains growing with interfering root systems may show differences of as much as 10 : 7 in the salinity of their cell sap. This fact, he suggests, may have some utility in the breeding of strains for the salty lands of the northern delta of Egypt.

In conclusion, Ball makes the significant suggestion: "Egyptian cotton (*Gossypium peruvianum*) may be classified as a facultative halophyte."

As far as we are aware the only previous work on the hydrogen-ion concentration of the tissue fluids of cotton is the single determination of P_H 4.6 in leaf, stem, and petal tissue given by Atkins (*1*). As will be shown later, this represents a distinctly higher acidity than that which we have found in any instance in the leaf tissue fluids of cotton.

MATERIALS AND METHODS

Since we are here dealing with a problem in which every possible source of error must be considered, and in as far as possible eliminated, in order to secure conclusive results, we shall describe the materials and methods employed in some detail. The importance of these details is not, we believe, overemphasized.

A detailed discussion of the Egyptian and Upland types of cotton and of their F_1 hybrid is not necessary in this place in view of the fact that the results of an investigation of the hybrids which has been in progress for the past several years have been published (*40*). The data given by Kearney in regard to height of plants and dimensions of the leaves were confirmed by measurements made on the material used by the writers in their investigations in 1921. It need only be mentioned here that the Egyptian and Upland types differ in a large number of morphological characters, the differences being of a kind and degree indicating that they belong to very distinct botanical species. When the two types are grown under comparable conditions the Egyptian plant is taller and has longer internodes and branches and larger, more deeply lobed, thicker, smoother, and darker green leaves than the Upland plant.

GENETIC NATURE OF THE SEED EMPLOYED, AND CHARACTERISTICS OF THE VARIETIES AND OF THEIR F_1 HYBRID

Three varieties of cotton are considered in this paper: Pima represents the Egyptian, while Acala and Meade belong to the Upland group of cottons.

The following is the history of the Egyptian and Upland seed employed for the special cultures upon which the physicochemical determinations were made in 1921. The origin and characteristics of the Pima variety have been described by Kearney (*37*, *40*). The "Pima selfed" seed was obtained by selfing a number of plants in a progeny designated Pima H1 – 5 Acala A, which was grown at Sacaton in 1920. This progeny was derived, by three generations of controlled self-pollination, from progeny P1-40 of 1917, which had been derived by three generations of controlled self-pollination, from plant P1, selected in 1914. The line of descent of P1-40 has been described elsewhere (*38*). The "Pima selfed" material of this experiment represented, therefore, seven generations of strict inbreeding and was presumably approaching homozygosity.

The origin and characteristics of the Meade variety are described by Meloy and Doyle (*46*).

The "Meade selfed" seed was secured by self-fertilizing a few plants of Meade cotton grown at Sacaton in 1920. These were grown from seed harvested from experimental plantings in Georgia, conducted under the direction of Dr. O. F. Cook, of the Office of Acclimatization and Adaptation Investigations of the Bureau of Plant Industry. This

seed came from isolated cultures of naturally pollinated (not selfed) plants. Mr. G. S. Meloy, who furnished the seed, considers it pure as far as varietal characters are concerned. It is not, however, as closely inbred as is the "Pima selfed" of the experiment.

The "Pima bulk" of the experiment was grown from seed from a selected but not strictly inbred strain. While unquestionably pure Pima, it was not of controlled, self-fertilized, ancestry.

The Meade bulk and the Acala [4] bulk used in the planting were both from lots of seed sent to Sacaton by Mr. Meloy in 1919 and held over until 1921. Both are supposed to be varietally pure, although not resulting from controlled inbreeding.

The F_1 hybrid was derived as follows: The Pima parent of the P × M F_1 used was a plant representing the sixth inbred generation in progeny Pima H1–2A of 1920, a sister progeny of PH1–5 Acala A which furnished the "Pima selfed" seed in the experiment.

The Meade parent of the P × M F_1 was a plant grown at Sacaton in 1920 from seed obtained from the experimental plantings in Georgia. This plant was one of a lot grown from seed produced by naturally pollinated (not selfed) flowers in an isolated planting of this variety in Georgia, and was considered "pure" by Mr. Meloy, who sent it to Sacaton.

Flowers were selfed on a few individuals in the 1920 planting at Sacaton, and these furnished the "Meade selfed" seed planted in the physicochemical experiments of 1921. Thus the Meade parent of this hybrid was by no means so closely bred as the Pima parent.

It may be noted that the seed used was not of exactly the same age for all of the cultures. This difference in age probably does not influence the physiological characteristics of the plants in any way, since the cotton seed is very long lived.[5]

The F_1 hybrids between Egyptian and Upland cotton show intensification in most size characters. In respect to other characters the hybrid mean may be intermediate or may approximate that of one or the other parent. The F_1 is highly uniform. The greater vegetative vigor is conspicuously shown by the accompanying plate (Pl. 1), in which the F_1 hybrid is shown between the Egyptian and the Upland parental types.

CULTURAL METHODS

In preparing the planting scheme it seemed desirable to grow the plants under the standard conditions of the Cooperative Testing Station experiments with the cottons. This involved the use of an irrigation border 26.5 feet in width by 400 feet in length. This accommodated seven rows of cotton 3.5 feet apart, with plants 1 foot apart in the rows. In order to avoid the influence of soil heterogeneity, which has been shown to be an important factor in crop yields (*21*, *32*), and which may reasonably be assumed to be an important factor in determining the physicochemical properties of the tissue fluids of the plant organism in regions

[4] Acala is an Upland variety of Mexican origin, introduced by G. N. Collins and C. B. Doyle, and adapted by selection to conditions in the United States by O. F. Cook and his colleagues. Acala is a big-bolled type, producing fiber from 1 1/16 to 1 1/8 inches long.

[5] It has been found that Pima seed which had been stored at Sacaton during nine years germinated readily when planted. Moreover, the stock of Pima planting seed distributed to farmers in the Salt River Valley in the spring of 1921 had been held over one year at Tempe. In order to make sure that its viability was unimpaired, the germination was thoroughly tested by the seed laboratory of the Bureau of Plant Industry just before the seed was distributed, and the average germination percentage proved to be exactly the same as that for the same stock of seed shortly after it was harvested. The seed was approximately one year older when the second series of germination tests were made. There is a possibility that seed of the Upland cottons may lose its viability more rapidly, but this is improbable.

in which the soil is rich in inorganic solutes, it seemed essential to distribute the Egyptian and Upland cottons and their F_1 hybrids uniformly over the field.

The plan adopted is shown in figure 1. Here the heavy line indicates Pima Egyptian, the broken line indicates Meade Upland, the light solid line indicates Acala Upland, and H denotes a hybrid plant. Beginning at the south end of the field, 10 feet of each row was planted to Pima cotton. On four of the rows (1, 3, 5, and 7) one hill of hybrid was planted. On the first and fifth row self-fertilized Pima and Meade seed was used. For the other rows bulk seed was employed.

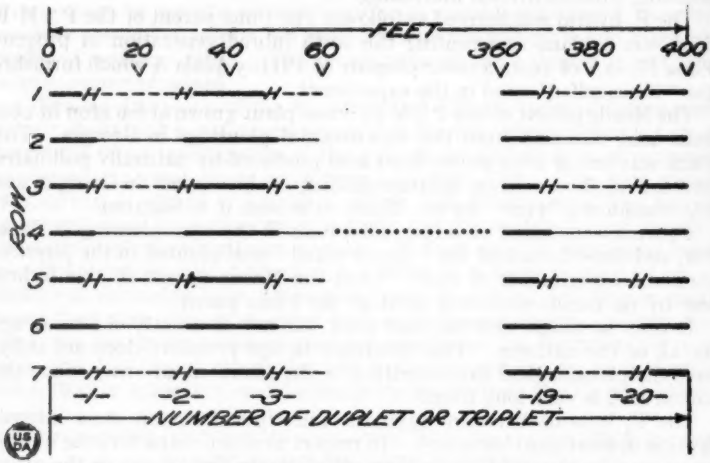

FIG. 1.—Planting scheme for comparison of Upland and Egyptian cottons and their F_1 hybrid. Only the two ends of the plot are shown. The heavy solid line represents plants of Pima Egyptian, the broken line plants of Meade Upland, the light solid line plants of Acala Upland, and the letter H an F_1 hybrid plant. The term duplet is used to designate plants of Egyptian and Upland which are contiguous and in the same row. The term triplet designates an F_1 plant (H) having Egyptian plants on one side and Upland plants on the other, all in the same row.

The Pima cotton and the hybrid, when it occurred, was then followed by 20 feet of Upland cotton, which was followed by 20 feet of Pima on rows 2, 4, and 6, and by a hybrid plant, and then by slightly less than 20 feet of Pima on rows 1, 3, 5, and 7. Thus the length of the plot afforded space in each row for 9 subrows, each of 20 feet, and for 2 half-subrows, each of 10 feet, of Pima cotton, and for 10 subrows, each of 20 feet, of Upland cotton. In the rows which contained hybrid plants the space for the Pima and Upland subrows was slightly shortened to make room for the hybrid hills. The spacing in the rows of the individual plants of all varieties and hybrids was identical.

This plan distributed plants of all types to be compared uniformly over the entire field. Collections were made at the 20 points of contact of Egyptian and Upland cotton or of Egyptian, hybrid, and Upland cotton in each of the rows. The hybrid plant and the contiguous Egyptian and Upland plants are designated as a triplet. The contiguous plants of Egyptian and Upland cotton in the rows containing no hybrid individuals are conveniently designated as a duplet.

The cultural technique was that generally employed for the growth of Egyptian and Upland cotton at the Cooperative Testing Station at Sacaton. Since all the materials considered were grown under as nearly as possible identical conditions, it seems unnecessary to detail these methods here.[6]

COLLECTION OF SAMPLES

Cotton is not in its morphological features a desert plant. It wilts readily and must be copiously supplied with irrigation water in order to produce a crop under the intense heat and scanty precipitation of the Southwest. During August the plants and the condition of the soil in which they are growing are continually varying, not merely from day to day but literally from minute to minute. Immediately after irrigation, in which the whole surface of the "border" is flooded with a sheet of water, the soil is fully saturated with water, but this is lost very rapidly. Water loss from the plants through transpiration increases very rapidly after sunrise, and great care must be taken not to obtain samples of leaves which have already begun to wilt. When there is a heavy dew in the morning, as occasionally occurs after the summer rains have begun, it is difficult to secure samples in the limited period between the time when the leaves have scattered droplets of external moisture and the time when they begin to wilt under the influence of the intense insolation.

Because of the continual changes in the condition of the plants, the taking of the samples is one of the most exacting features of the work. In addition to the care necessary in selecting the brief period most suitable for taking the samples, two possible sources of error had to be carefully avoided.

There must be introduced no artificial source of differentiation between the two species under consideration, or between the species and their hybrid, through the taking of the samples from different portions of the field or at different times.

The leaves taken from the plants to be compared must be comparable in maturity.

Artificial differentiation between the species or between the species and their hybrid may be avoided in cases in which the varieties to be compared belong to the same duplet (rows 2, 4, and 6) or the same triplet (rows 1 and 5 and rows 3 and 7) by making sure that collections are taken simultaneously from the two or three members of the duplet or triplet.

In the first series of collections (1920) it was possible to avoid the influence of heterogeneity in the soil conditions[7] because of the fact that in a number of breeding experiments under way at Sacaton, Pima cotton and Upland cotton varieties were grown side by side in the same plot or "border." All collections were taken from adjacent rows or from closely associated plants in the same row. In the investigations of 1921 the possible influence of this factor was precluded by a special planting scheme, as described above.

To avoid the possible influence of time variations, the samples from the two or three members of the duplet or triplet were taken at as

[6] We are greatly indebted to Mr. Walter F. Gilpin, who carefully made the plantings, took some of the records and facilitated our work in other ways.

[7] Readings made by Mr. Kearney with the soil bridge on samples of soil taken in the neighborhood of the various sets of tissue samples, indicated differences in the salt content from place to place in the field.

nearly as possible the same time. Probably not more than five minutes elapsed in any case between the sampling of any two members of a group. Generally the time was much less than this. Probably no appreciable source of error was introduced in this way.

In the case of the loss of any sample through breakage, contamination, or through any discernible evidence of an erroneous reading, new samples of both members of the duplet or of all three members of the triplet were taken before the completion of the whole series of determinations.

Rows 1 and 5 were planted to self-fertilized Pima and self-fertilized Meade. Rows 3 and 7 were planted to Pima and Meade grown from bulk seed. Thus it is possible to consider in a preliminary way whether there are differences between the sap properties of these two classes (those grown from self-fertilized and those grown from open-fertilized seed) of plants. Again, rows 1, 3, 5, and 7 were planted to Pima Egyptian and to the Upland variety Acala. Thus it is possible to compare the two varieties of Upland cotton in a wholly preliminary way.

If the samples had been taken systematically across the field, it is evident that changing conditions from day to day might be a source of large differences between the several rows. The collections were, therefore, made from duplets or triplets selected as nearly as possible at random from different portions of the field.

In order to avoid in so far as possible differences due to age, collections were made primarily of matured leaves from the main stem or the largest branches of the plants. Great care was taken to select perfect leaves.

A possible source of variation in the constants is personal equation in the making of the collections. The taking of the samples presented considerable difficulty because of the limited time in which it was necessarily carried out in order to have environmental conditions as nearly comparable as possible. Three workers, therefore, took part in collecting the material.

In the first series of collections the samples were taken by two workers, and notes were taken by the third. Had one of the two workers taken either Egyptian, Upland, or hybrid samples continuously, it seems quite possible that differences in personal equation might have influenced the results. But the two collectors generally alternated in the variety collected. Thus, any systematic influence of personal equation was avoided.

It is evident, however, that if there be a real personal equation on the part of the different collectors, this will tend, in the system of collection adopted for the first series, to obscure the difference between the forms.

To avoid this possibility, a modification of the plan was adopted in the second series of collections. One worker, who had enjoyed longer experience in botanical work than the others, collected all the leaves used for samples. Thus the possibility of an influence of personal equation in the collection of the samples would seem to have been practically, if not entirely, eliminated in the second series.

ANALYTICAL METHODS

The samples of tissue were placed at once in rubber-stoppered glass tubes and in a very short time were in a cooling mixture for freezing antecedent to the extraction of the sap, in accordance with the findings of

Dixon and Atkins (*12*) and ourselves (*16*, *17*). Freezing-point lowering, Δ; specific electrical conductivity, κ; and hydrogen-ion concentration, expressed as P_H, were then determined on the centrifuged sap, extracted from the tissue by pressure.

In the first series of determinations (see p. 283) the physical measurements were made as soon as possible after the extraction of the leaf fluids. In the second series the expressed sap was allowed to stand overnight in the ice box.

Freezing point lowering was measured by means of a mercury thermometer graduated to hundredths of degrees. Electrical conductivity was determined at 30° in Freas conductivity cells standardized against *N*/10 KCl, considered as having a conductivity of 0.01412 reciprocal ohm at 30°. The Washburn half-meter bridge with extension coils, giving 10 M. of wire, and a Leeds and Northrup resistance box was used.

Hydrogen-ion concentration was measured by means of the Wendt modification of the Hildebrand assemblage. In this method a calomel cell charged with normal KCl, a small Weston Millivoltmeter and a sliding resistance of 500 ohms with current capacity of one ampere were used. Hildebrand, Sharp, Hoagland, and others (*10*) have recognized the limitations of precision of this method. The accuracy of the physical measurements is, however, sufficient for the comparisons which are emphasized in this paper.

In discussing these acidities we must not forget that the determinations were made on sap extracted by pressure from killed tissues. Haas (*18*) has stated that a considerable change in reaction may take place in the cell as it dies (from P_H 3 to P_H 7), but he evidently refers to the changes taking place in the natural ageing and death of the cells, rather than to errors introduced by the killing of the cell and the extraction of the cell sap. His own determinations were made by means of extracts obtained by rapidly macerating petals in a mortar with the solvent. McClendon and Sharp (*44*) noted an increase in the acidity of freshly expressed carrot juice on exposure to the air for a short time. They also give some data on P_H of fluids extracted from boiled and unboiled tissues. Finally, Atkins (1) has noted a similar increase in the acidity of expressed sap on exposure to the air, and suggests a coordination of this result with those obtained by Bunzell (*8*) on acidity and oxidase activity. It is also possible, as noted by Hoagland and Davis (*35*), that extracellular substances in the tissues may influence the concentration or reaction of the cell sap after the crushing of the tissues.

Whatever may be the results of further investigations on the relative values of the hydrogen-ion concentration of extracted sap as compared with that of the sap occurring in the tissues, the facts remain (*a*) that the only method by which the problem of hydrogen-ion concentration may be investigated in a series like the present is upon extracted sap, (*b*) that the technique used introduces no artificial differences between the biological series considered, and (*c*) that, as will be shown below, the results for the various series considered here show a high degree of consistency, thus indicating that there is no highly variable disturbing factor in the technique.

The results in the accompanying tables are expressed in terms of freezing-point depression, Δ, corrected for the amount of ice separating on under-cooling. Those who prefer to consider the results in terms of atmospheres may transmute the values of freezing-point lowering given

in this paper into atmospheres by the use of a published table (*24*), as we have done in a few cases.

Conductivities have been expressed in reciprocal ohms or mhos.

At the time of making these determinations we were unacquainted with the methods suggested by Mason (*45*) for estimating the influence of viscosity upon electrical conductivity. The conductivities given are, therefore, the raw values wholly uncorrected for the resistance of the sap. Inasmuch as the biological forms considered do not show large percentage difference in freezing-point depression, this fact probably does not invalidate the constants to a measurable degree for the purpose in hand.

We have felt it desirable to express in some manner the relative value of the electrical conductivity.

While Chandler (*9*), Dixon, and Atkins (*13*), and more recently Mason (*45*), have attempted to differentiate between electrolytes and nonelectrolytes by expressing the conductivity in terms of the osmotic concentration which would be produced by a solution of a specific solute (KCl) of the same electrical conductivity as the sap, it has seemed to us wisest to limit ourselves, as in earlier papers, to the purely numerical ratio of one constant to the other. We have, therefore, merely employed the ratio of the specific electrical conductivity to the freezing point depression as a measure of the relative concentration of dissociated ions and total solutes (both dissociated and nondissociated electrolytes and nonelectrolytes). It is impossible to attach any specific chemical meaning to the ratio κ/Δ. It is, however, useful for purposes of comparison.

The values of hydrogen-ion concentration have been calculated by the formula

$$P_H = \log \frac{1}{[H]} = \frac{E' - E}{0.0001983\ T}.$$

where E' is the electromotive force in millivolts of the tissue fluid under investigation as read from the voltmeter, E is the E. M. F. of the KCl calomel electrode employed (taken as $E = 0.2828$ volt for the normal KCl electrode), and T the absolute temperature.[8]

STATISTICAL ANALYSIS OF DATA

In the analysis of the series of measurements it has been necessary to employ to some extent the methods of the modern higher statistics. The simpler biometric notation is now familiar to most biologists. When special methods are used, formulas are given.

PRESENTATION AND ANALYSIS OF DATA FOR THE COMPARISON OF PARENTAL TYPES

Because of the large number of factors which might influence the results of physicochemical measurements on the tissue fluids of a crop plant such as cotton, and because of the fact that the varieties considered in 1920 and 1921 are not in all cases the same, it seems desirable to present the results of the preliminary investigation carried out by two of us in 1920, as well as the results of the more carefully planned investigation of 1921.

[8] We are deeply indebted to Mr. C. J. King, who encouraged us to monopolize practically the whole of his laboratory space while our work at Sacaton was in progress and facilitated our work in every way in his power.

PRELIMINARY STUDY IN 1920

A first comparison was based on Pima Egyptian and Acala Upland cotton taken August 14, 1920. The plants from the north and south ends of this border differed greatly in size, apparently because of the influence of alkali. Samples 1 and 2 were from well-grown plants from the south ends of the border. Samples 3 and 4 were from somewhat dwarfed plants at the north end of the border.

TABLE I.—*Physicochemical constants for the leaf tissue fluids of various types of cotton grown in 1920, and the differences between the constants for the various types*

Types and varieties compared.	Sample numbers.	Depression of freezing point, Δ.	Osmotic concentration in atmospheres, P.	Specific electrical conductivity, κ.	Ratio of conductivity to depression, κ/Δ.
Pima Egyptian	(1)	1.392	16.74	0.03880	0.02787
Acala Upland	(2)	1.218	14.65	.03083	.02532
	(1)-(2)	+0.174	+2.09	+.00797	+.00255
Pima Egyptian	(3)	1.573	18.91	.03490	.02219
Acala Upland	(4)	1.349	16.23	.02635	.01953
	(3)-(4)	+0.224	+2.68	+.00855	+.00266
Pima Egyptian	(5)	1.244	14.97	.03071	.02468
Acala Upland	(6)	1.201	14.45	.02871	.02392
	(5)-(6)	+0.043	+0.52	.00200	+.00076
Pima Egyptian	(7)	1.390	16.72	.03152	.02266
Sea Island	(8)	1.193	14.35	.02930	.02456
Acala Upland	(9)	1.274	15.33	.03040	.02386
Kekchi Upland	(10)	1.178	14.17	.02939	.02496
	(7)-(8)	+0.197	+2.37	+.00222	−.00190
	(7)-(9)	+0.116	+1.39	+.00112	−.00120
	(7)-(10)	+0.212	+2.55	+.00213	−.00230
	(8)-(9)	−0.081	−0.98	−.00110	+.00070
	(8)-(10)	+0.015	+0.18	−.00009	−.00040
Pima Egyptian	(11)	1.432	17.22	.03184	.02224
Wa Gale Asiatic	(12)	1.169	14.06	.02839	.02430
	(11)-(12)	+0.263	+3.16	+.00345	−.00206
Pima Egyptian	(13)	1.285	15.46	.04781	.03721
Lone Star Upland	(14)	1.058	12.74	.03612	.03415
	(13)-(14)	+0.227	+2.72	+.01169	+.00306
Pima Egyptian	(15)	1.123	13.51	.03661	.03260
Lone Star Upland	(16)	0.943	11.35	.03188	.03471
	(15)-(16)	+0.180	+2.16	+.00473	−.00211
Pima Egyptian	(17)	1.334	16.05	.03461	.02594
Lone Star Upland	(18)	0.974	11.72	.02788	.02864
	(17)-(18)	+0.360	+4.33	+.00673	−.00270
Pima Egyptian	(19)	1.154	13.89	.02971	.02575
Durango Upland	(20)	1.030	12.39	.02656	.02580
	(19)-(20)	+0.124	+1.50	+.00315	−.00005
Pima Egyptian	(21)	1.254	15.09	.03190	.02543
Durango Upland	(22)	1.230	14.80	.03118	.02536
	(21)-(22)	+0.024	+0.29	+.00072	+.00007
Pima Egyptian	(23)	1.162	13.98	.03163	.02724
Durango Upland	(24)	1.055	12.70	.02861	.02712
	(23)-(24)	+0.107	+1.28	+.00302	+.00012
Pima Egyptian	(25)	1.347	16.20	.05574	.04137
Holdon Upland	(26)	1.250	15.04	.05154	.04123
	(25)-(26)	+0.097	+1.16	+.00420	+.00014
Pima Egyptain	(27)	1.253	15.07	.03209	.02561
Acala Upland	(28)	1.113	13.40	.02794	.02510
	(27)-(28)	+0.140	+1.67	+.00415	+.00051

A second series of samples was taken on August 18. Sample 6 represents Acala Upland cotton growing between two rows of Pima Egyptian (sample 5). On this date it was feasible to secure samples of Pima Egyptian (7), Sea Island (8), Acala Upland (9), and Kekchi Upland (10) in close proximity in the same border. Pima Egyptian is compared with all the other types, and Sea Island is compared with the two Upland varieties. A comparison of Pima Egyptian (sample 11) with *Gossypium herbaceum* var. Wa-Gale (sample 12) from the Transcaucasian region was also made on this date.

On August 22 a third series of comparisons was made with the following samples from the yield test plots of Pima Egyptian, Lone Star Upland, and Durango Upland. In the comparisons of Pima Egyptian and Lone Star Upland cotton, samples 13–14 were taken near the south end of the border, samples 15–16 near the middle, and samples 17–18 near the north end. In the same test plot and on the same date a comparison of Pima with Durango was also made on the basis of samples near the south end (19–20), near the middle (21–22), and near the north end (23–24) of the border. In another border we took Pima Egyptian and Holdon Upland for comparison with the F_1 hybrid between the two species. (Samples 25–26.) For constants for the hybrid see sample 34, p. 299. In the same border we took Pima Egyptian (27) and Acala Upland(28) for comparison with an F_1 hybrid. (See No. 35, elsewhere.)

The constants for the individual samples are compared in Table I.

Comparing the values of freezing-point lowering and calculated osmotic pressure in Pima Egyptian cotton with the 13 determinations [9] on the several varieties of Upland cotton, we note that without exception the osmotic concentration is higher in the Egyptian cotton than in the Upland types.

Calculating the statistical constants for freezing-point lowering, Δ, in Pima and Upland, the data presented in Table II were obtained, the mean being the average value of the constant, S. D. the standard deviation, C. V. the coefficient of variation, and r, the coefficient of correlation between the two variables denoted by the subscript.

TABLE II.—*Statistical constants for freezing-point lowering in Pima and Upland cottons in 1920*

	Pima, *P.*	Upland, *U.*
Mean	1.300±0.022	1.144±0.022
S. D.	0.118±0.016	0.120±0.016
C. V.	9.100	10.456

$r_{PU}=+0.7439\pm0.0836$

In determining the probable error of the difference we must remember that because of field and meteorological heterogeneity (*21*) the values of osmotic pressure in associated plants of Pima and Upland types may be correlated. As a matter of fact this correlation is found to be $r=0.744$. This shows that the difference in salt content of the soil or differences in

[9] In one case (No. 7) a single sample of Egyptian cotton is compared with two varieties closely associated in the same plot of Upland cotton. In calculating the statistical constants for the whole series of each type, this one sample of Egyptian cotton is therefore used twice.

the time of the collection of the different samples influence in a somewhat similar manner the sap properties of both Pima and Upland types. The standard deviation of the difference between the constants of the two species is given by the formula

$$\sigma^2_{(P-U)} = \sigma^2_P + \sigma^2_U - 2r_{PU}\,\sigma_P\,\sigma_U.$$

Using this formula, we see that the difference in freezing point lowering of the tissue fluids of Egyptian and Upland cotton is 0.1560 ± 0.0159.

This value is 9.8 times as large as its probable error. While the number of samples is small it seems clear that, at this particular growth stage at least, the osmotic concentration of the leaf-tissue fluids of the Egyptian cotton is distinctly greater than that of the Upland. The fact that the individual differences are without exception of the same sign is a strong evidence for the validity of this conclusion.

The results for osmotic pressure, P, are stated in Table III. The difference in mean osmotic concentration is 1.872 ± 0.191 atmospheres.

TABLE III.—*Statistical constants for osmotic pressure in Pima and Upland cottons in 1920*

	Pima, *P.*	Upland, *U.*
Mean	15.639 ± 0.265	13.767 ± 0.269
S. D.	1.419 ± 0.188	1.437 ± 0.190
C. V.	9.074	10.44

$$r_{PU} = +0.7436 \pm 0.0836$$

The specific electrical conductivities, κ, are without exception somewhat higher in Pima than in the varieties of Upland cotton with which it is compared. Calculating the statistical constants for electrical conductivity and determining the probable errors by the method above, the data presented in Table IV were obtained. The difference in the electrical conductivity is 0.00463 ± 0.00058, showing that the tissue fluids of the Egyptian cotton contain somewhat larger quantities of ionized electrolytes than do those of Upland varieties.

TABLE IV.—*Statistical constants for electrical conductivity in Pima and Upland cottons in 1920*

	Pima, *P.*	Upland, *U.*
Mean	0.03596 ± 0.00137	0.03133 ± 0.00119
S. D.	0.00733 ± 0.00097	0.00633 ± 0.00084
C. V.	20.39	20.21

$$r_{PU} = +0.9058 \pm 0.0336$$

Comparing the values of the ratio of electrical conductivity to freezing point lowering, κ/Δ, for the 13 pairs of Egyptian and Upland cotton, we find that in eight cases the ratio is higher in the Egyptian than in the Upland cottons, whereas in five cases the results indicate that the relative proportion of conducting electrolytes is lower in the Egyptian than in

the Upland cottons. The differences in the ratios are relatively small. The average of the eight differences which indicate higher ratios of κ to Δ in the Egyptian cotton is 0.00123, whereas the average of the five cases which indicate higher ratios of κ to Δ in the Upland cotton is −0.00167. The general average for the 13 differences is 0.00012 ± 0.00034. Here the probable error has been determined by the formula given above, the statistical analysis having given the constants presented in Table V.

TABLE V.—*Statistical constants for the ratio of electrical conductivity to freezing point lowering in Pima and Upland cottons in 1920*

	Pima, *P.*	Upland, *U.*
Mean	0.02779 ± 0.00105	0.02767 ± 0.00104
S. D.	0.00560 ± 0.00074	0.00555 ± 0.00073
C. V.	20.17	20.04

$$r_{PU} = +0.9479 \pm 0.0189$$

Turning back to a comparison of the Egyptian with cottons other than the Upland, we note from samples 7 and 8 that the osmotic pressures and electrical conductivities are higher in Pima than in Sea Island cotton but that the ratio of electrical conductivity to freezing-point lowering is lower in Pima. Samples 11 and 12 show the same relationship to hold in a comparison of Pima Egyptian with the Transcaucasian *Gossypium herbaceum* var. Wa-Gale.

While these two cases taken alone are altogether inadequate as bases for conclusion concerning differences between Pima Egyptian cotton and other species of the genus Gossypium, it is to be noted that the differences are of precisely the same kind as are found in the comparison of the Egyptian and Upland types.

The results, therefore, strengthen the conclusion to be drawn from a comparison of Egyptian and Upland cottons, and suggest the interest of a more extensive comparative study of the different cotton species.

INVESTIGATIONS IN 1921

The organization of the experimental details of the work carried out in 1921 has been fully described above. It remains merely to state that two complete series of determinations were made and to note certain differences in the conditions of these series.

The sampling of the culture was begun on August 6, and the first series of samples—80 triplets and 60 duplets, 360 samples in all, less a few for which plants were not available—was completed on August 16.

At the time the first series was taken the soil moisture was ample but the surface layer was not wet. The plants at the beginning were in a rapidly growing condition, with but few bolls developing. Their growth was, however, being checked by the inadequacy of soil moisture when the collections were completed.[10]

The plants were given an ample irrigation immediately after the last samples were taken. After this irrigation there was an unusually

[10] Irrigation was delayed somewhat beyond the time at which water might advantageously have been applied in order that the first series of determinations might be completed in the period between two irrigations.

heavy rain. This left the fine silt in such a state that collections could not be taken up for the second series until August 19. The second series was completed on August 27.

In the first series collections were made from the single Egyptian and the single Upland plant adjoining the hybrid plant or adjoining each other. This was done in order to obtain the samples from plants subjected to as nearly identical environmental conditions as possible.

The method of collection followed in the first series has the disadvantage that the materials upon which the physical constants are to be based are drawn from single individuals, and are therefore subject to the variations characteristic of individual plants.

In taking up the second series of collections, it was evident that the two plants of Egyptian and Upland cotton from which the first samples had been taken were so depleted of the more mature leaves that it would be desirable to extend the range of individuals somewhat, and to include the adjoining plants in the taking of the samples representing the two parent forms. This could not be done in the case of the hybrid, which was represented by but a single individual in each triplet, but there was little difficulty in obtaining adequately large samples from the F_1 hybrid plants, which were larger than the others.

When the second series of determinations was taken up, it seemed desirable to make all the readings in duplicate. Time was available for this extra safeguard because of the standardization of every detail of the laboratory routine which had been worked out in the first series of determinations. The conductivities were reread, frequently with a different resistance; the freezing-point determinations were repeated on the same sample of fluid, thawed after the first freezing; and the hydrogen-ion determinations were made in duplicate, or repetition readings were made with the same electrodes. The average of these duplicate determinations have been used in the final calculations of the statistical constants. Except for a very few cases in which there was an obvious slip of the pen in recording the reading, the results of the first and second determinations check remarkably well.

Limitation of space precludes the publication of the individual determinations, nearly 3,000 in number, upon which our conclusions are based. It is necessary, therefore, to summarize the determinations by treating them statistically.

This may be done in two ways: First, the determinations may be arranged in an orderly manner and the frequency distribution of the magnitudes considered; second, statistical constants may be determined for the various subseries, and conclusions based on the comparison of these statistical constants, with due regard to their probable errors.

We have first of all to consider the groupings of the materials for seriation and for the determination of statistical constants. While the constants are available for the duplets or triplets of the individual rows, it does not seem expedient for present purposes to seriate the constants by rows, or to determine the statistical constants for such a detailed grouping of the materials. We have therefore combined the rows according to variety and origin of seed.[11] Thus rows 1 and 5, rows 3 and 7, and rows 2, 4, and 6 have been combined.

[11] In working with the original records for another purpose we have been careful to note that the conclusions drawn for the combined materials of the individual varieties are in general substantiated by the results for the individual rows. Because of the influence of the number of determinations on the probable errors of random sampling, the constants for the individual rows will show greater irregularity than those based on the combined records of two or more rows.

These represent the fundamental units on which the conclusions are based. Since rows 1 and 5 and 3 and 7 do not differ in variety but only in the origin of the seed used, the four rows (1, 3, 5, and 7) have been combined for a more extensive comparison of Pima and Meade cotton.

This has given a series of semi-independent subsamples. The concordance of the results from these subsamples is one of the strongest arguments for the validity of the conclusions based on the series as a whole.

In comparing the differences between the parental types, or between the parental types and the hybrids, we must remember that the two or three determinations on the same duplet or triplet will be rendered more or less alike by (*a*) the heterogeneity of the plot upon which the plants were grown and by (*b*) the differences in the time at which the samples were taken. Thus we should expect a correlation between the constants of the individuals of the same duplet or triplet, notwithstanding the fact that these individuals belong to different varieties of cotton and are merely rendered similar by community of environmental influences. The consequence of these correlations for the arguments of the present section of the investigation is that the probable error of the differences between the two parental types, and between the two individual parental types and the hybrid form, will be greatly reduced. We have, therefore, always calculated the probable error of the difference between the series of constants with due regard to the intensity of correlation between them.

In the analysis of the data we may first present the frequency distributions for the physicochemical measurements made. We shall then proceed to a more detailed comparison of the physicochemical constants by means of statistical constants.

We may now consider the frequency distributions of the constants for the individual classes of plants. The depression of the freezing point may be expressed in units of 5 per cent of the molecular lowering taken as $\Delta = 1.86$. The results are given in Table VI.

Inspection of these distributions shows clearly that the constants for Pima are somewhat higher than those for Meade. The numbers are rather too small to indicate clearly the nature of the distribution. In some cases they are wholly skew, but in general there is a suggestion of a more or less symmetrical distribution about a central mode. The frequencies for the hybrid suggest a lower minimum than that found in either of the parent forms.

The distribution of the measures of specific electrical conductivity is shown in Table VII. The distribution of the ratios of specific electrical conductivity to the freezing point lowering is represented in Table VIII.

The results for specific electrical conductivity show that the Egyptian cotton has a somewhat higher maximum than that found in the comparable Upland series. It is difficult to decide merely from inspection of the distributions the relative value of the ratio κ/Δ in the Upland, in the Egyptian, and in the F_1 forms.

The frequencies for the hydrogen-ion concentrations are shown in Table IX. Apparently the values of P_H are somewhat higher in the Upland than in the Egyptian form. The relation of the hybrid to the two parental forms can not be determined by inspection merely.

TABLE VI.—*Distribution of magnitudes of depression of freezing point, Δ, in the various comparisons of Pima Egyptian, Meade and Acala Upland, and F_1 hybrid cotton in the first and second series of determinations in 1921*

Type and variety of cotton and nature of seed planted.	Rows.	Series or collections.	0.884 to 0.976.	0.977 to 1.069.	1.070 to 1.162.	1.163 to 1.255.	1.256 to 1.348.	1.349 to 1.441.	1.442 to 1.534.	1.535 to 1.627.	1.628 to 1.720.	1.721 to 1.813.	1.814 to 1.906.	Total.
Pima Egyptian, from self-fertilized seed	1,5	First (1)	.....	.....	.....	11	8	3	9	3	4	.....	1	39
Meade Upland, from self-fertilized seed	1,5	First (1)	.....	.....	1	10	10	7	6	5	.....	.....	.....	39
F_1 hybrid	1,5	First (1)	.....	.....	7	7	12	6	3	4	.....	.....	.....	39
Pima Egyptian, from self-fertilized seed	1,5	Second (2)	.....	.....	3	12	13	5	5	1	.....	.....	.....	39
Meade Upland, from self-fertilized seed	1,5	Second (2)	.....	.....	15	12	5	7	.....	.....	.....	.....	.....	39
F_1 hybrid	1,5	Second (2)	1	9	16	6	6	1	.....	.....	.....	.....	.....	39
Pima Egyptian, from bulk seed	3,7	First (1)	.....	.....	1	4	15	9	8	1	.....	.....	.....	38
Meade Upland, from bulk seed	3,7	First (1)	.....	.....	2	7	8	11	10	.....	.....	.....	.....	38
F_1 hybrid	3,7	First (1)	.....	.....	3	8	18	7	2	1	.....	.....	.....	38
Pima Egyptian, from bulk seed	3,7	Second (2)	.....	.....	6	13	14	4	1	.....	.....	.....	.....	38
Meade Upland, from bulk seed	3,7	Second (2)	.....	.....	15	16	6	1	.....	.....	.....	.....	.....	38
F_1 hybrid	3,7	Second (2)	2	14	12	7	3	.....	.....	.....	.....	.....	.....	38
Pima Egyptian, combined series	1,3,5,7	First (1)	.....	.....	1	15	23	12	17	4	4	.....	1	77
Meade Upland, combined series	1,3,5,7	First (1)	.....	.....	3	17	18	18	16	5	.....	.....	.....	77
F_1 hybrid, combined series	1,3,5,7	First (1)	.....	.....	10	15	30	13	5	4	.....	.....	.....	77
Pima Egyptian, combined series	1,3,5,7	Second (2)	.....	.....	9	25	27	9	6	1	.....	.....	.....	77
Meade Upland, combined series	1,3,5,7	Second (2)	.....	.....	30	28	11	8	.....	.....	.....	.....	.....	77
F_1 hybrid, combined series	1,3,5,7	Second (2)	3	23	28	13	9	1	.....	.....	.....	.....	.....	77
Pima Egyptian, from bulk seed	2,4,6	First (1)	.....	.....	3	13	20	13	6	.....	4	.....	.....	59
Acala Upland, from bulk seed	2,4,6	First (1)	.....	.....	5	21	14	13	4	1	1	.....	.....	59
Pima Egyptian, from bulk seed	2,4,6	Second (2)	.....	.....	5	22	15	17	.....	.....	.....	.....	.....	59
Acala Upland, from bulk seed	2,4,6	Second (2)	.....	7	20	18	11	3	.....	.....	.....	.....	.....	59

TABLE VII.—*Distribution of magnitudes of specific electrical conductivity, $\kappa \times 10^8$, in the various comparisons of Pima Egyptian, Meade and Acala Upland, and F_1 hybrid cotton in the first and second series of determinations in 1921*

Type and variety of cotton and nature of seed planted.	Rows.	Series or collections.	21501 to 22500.	22501 to 23500.	23501 to 24500.	24501 to 25500.	25501 to 26500.	26501 to 27500.	27501 to 28500.	28501 to 29500.	29501 to 30500.	30501 to 31500.	31501 to 32500.	32501 to 33500.	33501 to 34500.	34501 to 35500.	35501 to 36500.	36501 to 37500.	37501 to 38500.	38501 to 39500.	39501 to 40500.	40501 to 41500.	41501 to 42500.	Total.
Pima Egyptian, from self-fertilized seed	1, 5	First (1)	....	....	....	....	....	....	1	2	1	8	7	1	6	6	2	3	....	1	....	....	1	39
Meade Upland, from self-fertilized seed	1, 5	First (1)	....	....	....	....	....	....	3	3	6	4	6	4	6	3	3	1	....	....	....	....	....	39
F_1 hybrid	1, 5	First (1)	....	....	....	....	....	....	2	9	7	10	1	5	1	1	....	1	....	....	....	....	....	39
Pima, Egyptian, from self-fertilized seed	1, 5	Second (2)	....	....	....	1	....	1	2	9	7	10	1	5	1	1	....	1	....	....	....	....	....	39
Meade Upland, from self-fertilized seed	1, 5	Second (2)	....	....	....	....	2	....	4	6	8	6	8	....	2	2	....	1	....	....	....	....	....	39
F_1 hybrid	1, 5	Second (2)	....	....	....	5	6	6	7	5	4	3	3	....	....	....	....	....	....	....	....	....	....	39
Pima Egyptian, from bulk seed	3, 7	First (1)	....	....	....	1	11	7	5	8	3	1	2	....	1	....	....	....	....	....	....	....	....	39
Meade Upland, from bulk seed	3, 7	First (1)	....	....	....	1	....	....	3	3	4	3	7	3	7	3	3	1	....	....	....	....	....	38
F_1 hybrid	3, 7	First (1)	....	....	....	1	....	1	4	4	3	8	2	8	5	2	....	....	....	....	....	....	....	38
Pima Egyptian, from bulk seed	3, 7	Second (2)	....	....	....	1	1	....	2	6	8	7	6	4	2	1	....	....	....	....	....	....	....	38
Meade Upland, from bulk seed	3, 7	Second (2)	....	....	....	....	....	2	6	5	11	5	3	3	....	....	....	....	....	....	....	....	....	38
F_1 hybrid	3, 7	Second (2)	....	1	2	1	8	6	11	5	3	1	....	....	....	....	....	....	....	....	....	....	....	38
Pima Egyptian, combined series	1, 3, 5, 7	First (1)	1	....	4	4	9	5	4	7	2	2	....	....	....	....	....	....	....	....	....	....	....	38
Meade Upland, combined series	1, 3, 5, 7	First (1)	....	....	....	1	....	....	4	5	5	11	14	4	13	9	5	4	....	1	....	....	1	77
F_1 hybrid, combined series	1, 3, 5, 7	First (1)	....	....	....	1	....	1	7	9	12	8	12	11	5	3	1	....	....	....	....	....	....	77
Pima Egyptian, combined series	1, 3, 5, 7	First (1)	....	....	....	2	1	1	4	15	15	17	7	9	3	2	....	1	....	....	....	....	....	77
Meade Upland, combined series	1, 3, 5, 7	Second (2)	....	....	....	....	4	3	10	11	19	11	11	3	2	2	....	1	....	....	....	....	....	77
F_1 hybrid, combined series	1, 3, 5, 7	Second (2)	....	1	2	6	14	12	18	10	7	4	3	....	....	....	....	....	....	....	....	....	....	77
Pima Egyptian, from bulk seed	2, 4, 6	First (1)	1	....	4	5	20	12	9	15	5	3	2	....	1	....	....	....	....	....	....	....	....	77
Acala Upland, from bulk seed	2, 4, 6	First (1)	....	....	....	....	....	....	....	9	10	9	7	5	11	4	3	....	....	....	....	1	....	59
Pima Egyptian, from bulk seed	2, 4, 6	Second (2)	....	....	1	....	....	2	2	7	8	15	13	3	4	4	....	....	....	....	....	....	....	59
Acala Upland, from bulk seed	2, 4, 6	Second (2)	....	....	....	....	1	4	12	7	8	15	6	5	....	1	....	....	....	....	....	....	....	59
			....	....	2	8	5	13	12	10	4	5	....	....	....	....	....	....	....	....	....	....	....	59

TABLE VIII.—*Distribution of magnitudes of the ratio of specific electrical conductivity to the freezing point lowering, $\kappa/\Delta \times 10^6$, in the various comparisons of Pima Egyptian, Meade and Acala Upland, and F_1 hybrid cotton, in 1921, in the first and second series of determinations*

Type and variety of cotton and nature of seed planted.	Rows.	Series or collections.	17501 to 18500.	18501 to 19500.	19501 to 20500.	20501 to 21500.	21501 to 22500.	22501 to 23500.	23501 to 24500.	24501 to 25500.	25501 to 26500.	26501 to 27500.	27501 to 28500.	28501 to 29500.	Total.
Pima Egyptian, from self-fertilized seed	1, 5	First (1)	.....	.....	.....	3	5	10	7	4	9	1	.....	.....	39
Meade Upland, from self-fertilized seed	1, 5	First (1)	.....	.....	.....	1	5	12	9	6	6	.....	.....	.....	39
F_1 hybrid	1, 5	First (1)	.....	1	.....	2	5	12	11	2	1	2	2	1	39
Pima Egyptian, from self-fertilized seed	1, 5	Second (2)	.....	.....	1	4	5	4	16	5	2	.....	2	.....	39
Meade Upland, from self-fertilized seed	1, 5	Second (2)	.....	.....	1	3	7	12	9	4	2	1	.....	.....	39
F_1 hybrid	1, 5	Second (2)	.....	.....	.....	.....	2	3	12	14	5	3	.....	.....	39
Pima Egyptian, from bulk seed	3, 7	First (1)	.....	.....	.....	1	9	10	5	8	3	2	.....	.....	38
Meade Upland, from bulk seed	3, 7	First (1)	.....	2	1	4	8	4	8	9	1	1	.....	.....	38
F_1 hybrid	3, 7	First (1)	.....	1	.....	.....	6	9	13	4	4	.....	1	.....	38
Pima Egyptian, from bulk seed	3, 7	Second (2)	.....	.....	.....	1	7	9	14	5	1	1	.....	.....	38
Meade Upland, from bulk seed	3, 7	Second (2)	1	.....	2	4	1	15	10	5	.....	.....	.....	.....	38
F_1 hybrid	3, 7	Second (2)	.....	1	.....	1	1	7	7	13	7	1	.....	.....	38
Pima Egyptian, combined series	1, 3, 5, 7	First (1)	.....	.....	.....	4	14	20	12	12	12	3	.....	.....	77
Meade Upland, combined series	1, 3, 5, 7	First (1)	.....	2	1	5	13	16	17	15	7	1	.....	.....	77
F_1 hybrid, combined series	1, 3, 5, 7	First (1)	.....	2	.....	2	11	21	24	6	5	2	3	1	77
Pima Egyptian, combined series	1, 3, 5, 7	Second (2)	.....	.....	1	5	12	13	30	10	3	1	2	.....	77
Meade Upland, combined series	1, 3, 5, 7	Second (2)	1	.....	3	7	8	27	19	9	2	1	.....	.....	77
F_1 hybrid, combined series	1, 3, 5, 7	Second (2)	.....	1	.....	1	3	10	19	27	12	4	.....	.....	77
Pima Egyptian, from bulk seed	2, 4, 6	First (1)	.....	.....	1	1	5	16	17	12	6	1	.....	.....	59
Acala Upland, from bulk seed	2, 4, 6	First (1)	1	.....	.....	2	6	17	9	8	13	2	.....	1	59
Pima Egyptian, from bulk seed	2, 4, 6	Second (2)	.....	.....	2	1	8	23	14	7	3	1	.....	.....	59
Acala Upland, from bulk seed	2, 4, 6	Second (2)	.....	.....	.....	1	9	25	14	7	3	.....	.....	.....	59

TABLE IX.—*Distribution of magnitudes of the hydrogen-ion concentration, P_H, in the various comparisons of Pima Egyptian, Meade and Acala Upland, and F_1 hybrid cotton in the first and second series of determinations in 1921*

Type and variety of cotton and nature of seed planted.	Rows.	Series or collections.	4.976 to 5.025	5.026 to 5.075	5.076 to 5.125	5.126 to 5.175	5.176 to 5.225	5.226 to 5.275	5.276 to 5.325	5.326 to 5.375	5.376 to 5.425	5.426 to 5.475	5.476 to 5.525	5.526 to 5.575	5.576 to 5.625	5.626 to 5.675	5.676 to 5.725	Total.
Pima Egyptian, from self-fertilized seed	1,5	First (1)	.....	2	5	8	4	3	5	8	3	1	.....	.....	.....	.....	.....	39
Meade Upland, from self-fertilized seed	1,5	First (1)	.....	.....	1	1	4	5	5	10	8	.....	2	2	.....	1	.....	39
F_1 hybrid	1,5	First (1)	.....	1	.....	5	2	3	9	10	7	.....	.....	2	.....	.....	.....	39
Pima Egyptian, from self-fertilized seed	1,5	Second (2)	.....	.....	.....	2	1	6	7	12	7	3	1	.....	.....	.....	.....	39
Meade Upland, from self-fertilized seed	1,5	Second (2)	.....	.....	.....	.....	2	.....	3	1	6	11	7	3	6	.....	.....	39
F_1 hybrid	1,5	Second (2)	.....	.....	.....	.....	4	4	4	6	4	10	3	2	1	.....	1	39
Pima Egyptian, from bulk seed	3,7	First (1)	1	.....	2	5	5	7	7	7	2	2	.....	.....	.....	.....	.....	38
Meade Upland, from bulk seed	3,7	First (1)	.....	.....	.....	.....	3	4	3	8	5	6	4	4	.....	1	.....	38
F_1 hybrid	3,7	First (1)	.....	1	.....	6	3	6	6	8	4	3	1	.....	.....	.....	.....	38
Pima Egyptian, from bulk seed	3,7	Second (2)	.....	.....	.....	.....	1	10	4	7	1	7	3	4	1	.....	.....	38
Meade Upland, from bulk seed	3,7	Second (2)	.....	.....	.....	.....	2	.....	3	9	5	4	7	4	3	1	.....	38
F_1 hybrid	3,7	Second (2)	.....	.....	.....	1	1	5	7	4	5	5	4	5	.....	.....	1	38
Pima Egyptian, combined series	1,3,5,7	First (1)	1	2	7	13	9	10	12	15	5	3	.....	.....	.....	.....	.....	77
Meade Upland, combined series	1,3,5,7	First (1)	.....	.....	1	1	7	9	8	18	13	6	6	6	.....	2	.....	77
F^1 hybrid, combined series	1,3,5,7	First (1)	.....	2	.....	11	5	9	15	18	11	3	1	2	.....	.....	.....	77
Pima Egyptian, combined series	1,3,5,7	Second (2)	.....	.....	.....	2	2	16	11	19	8	10	4	4	1	.....	.....	77
Meade Upland, combined series	1,3,5,7	Second (2)	.....	.....	.....	.....	4	.....	6	10	11	15	14	7	9	1	.....	77
F_1 hybrid, combined series	1,3,5,7	Second (2)	.....	.....	.....	1	5	9	11	10	9	15	7	7	1	.....	2	77
Pima Egyptian, from bulk seed	2,4,6	First (1)	1	1	3	11	8	11	9	8	5	1	1	.....	.....	.....	.....	59
Acala Upland, from bulk seed	2,4,6	First (1)	.....	.....	2	6	6	10	12	12	6	4	1	.....	.....	.....	.....	59
Pima Egyptian, from bulk seed	2,4,6	Second (2)	.....	.....	.....	.....	3	1	9	10	11	15	5	2	1	2	.....	59
Acala Upland, from bulk seed	2,4,6	Second (2)	.....	.....	.....	.....	.....	2	5	5	14	15	7	2	4	4	1	59

These frequency distributions show two things:

The values of all the physicochemical properties investigated show considerable variability from sample to sample.

There are evidences of differences in the constants for the two parent forms and for the F_1 hybrid. It is clear, however, that for an analysis of these differences we must turn to a more careful study of statistical constants. Since, however, the utmost precision is required we have determined the constants directly from the ungrouped data. These have been recorded to four significant figures in the case of Δ, to five significant figures in the case of κ and κ/Δ, and to three significant figures in the case of P_H. These values are of course beyond the range of precision of the instruments employed, but they represent as close an approximation as it was possible to secure in the case of a single reading, and in the second series the results are, in practically all cases, the average of two readings.

COMPARISON ON THE BASIS OF FREEZING POINT DEPRESSIONS

The comparison between the various series of Pima Egyptian and Meade and Acala Upland cottons is made in Table X. Since all of the tables in which comparisons are drawn between the physicochemical properties of the Egyptian and Upland varieties are constructed in the same way, one explanation will suffice for those for κ, κ/Δ and P_H (Tables XI to XIII), as well as for Δ.

The varieties compared are given in column 1. For each variety two, and sometimes three, rows of plants are available.[12] By combining the Pima and Meade grown from self-fertilized and bulk seed respectively four rows are available for a comparison of Pima and Meade. In column 2 are given the numbers of the rows from which the samples were taken. Two sets of constants are available for each variety. The upper entry represents in each case the constants derived from the first series of determinations (August 6 to 16) while the lower entry represents the constants derived from the second series of determinations (August 19 to 27). Column 3 gives the number of individual determinations upon which the statistical constants are based. Column 4 indicates the series (i. e., the first or second collection) and the method of taking the differences in drawing the comparison between the constants for the earlier and for the later period. Column 5 gives the average value of freezing-point depression for Egyptian cotton; column 6 gives the comparable value for Upland cotton. Column 7 gives the correlation coefficient measuring the relationship between the constants of the plants, or small groups of plants, grown in immediate association in the various duplets or triplets. The purpose of these coefficients will be discussed presently.

Two sets of differences appear.

The first set, entered opposite the row numbers in columns 8–10, show the differences between Egyptian and Upland cotton. The differences in column 8 are so taken that a positive sign indicates a greater freezing point depression in the Egyptian than in the Upland cotton. These constants are provided with probable errors, calculated by the equation given above (p. 279). Column 9 shows the ratio of these differences to their probable errors.[13] The differences as given in column 8 are absolute

[12] The location of these rows in the field will be clear from the map, fig. 1, p. 272.

[13] These ratios have been calculated from the values of the differences and their probable errors carried to a larger number of places than can be given in the tables of this paper. They are, therefore, somewhat more accurate than those which may be recomputed from the values of the constants as given here.

TABLE X.—*Comparison of freezing point depression, Δ, in Pima Egyptian and Meade and Acala Upland cotton, and in the first and second series of determinations for each type, in 1921*

(1) Types and varieties compared.	(2) Rows.	(3) N	(4) Series or collection.	(5) Mean for Egyptian cotton.	(6) Mean for Upland cotton.	(7) Correlation between Egyptian and Upland.	Difference between Egyptian and Upland. (8) Absolute difference.	(9) Diff. / $E_{diff.}$	(10) Percentage difference.
Pima Egyptian and Meade Upland, from self-fertilized seed	1, 5	39	First (1)	1.4037±.0179	1.3528±.0137	+.8583	+.0509±.0093	5.46	3.76
	1, 5	39	Second (2)	1.2983±.0113	1.2122±.0107	+.7654	+.0861±.0076	11.4	7.10
			Diff. (2)−(1)	−.1054±.0165	−.1406±.0175	−.0929	+.0352±.0109	.....	.....
			Diff./$E_{diff.}$	6.39	8.06	.....	3.21	.....	.....
Pima Egyptian and Meade Upland, from bulk seed	3, 7	38	First (1)	1.3572±.0110	1.3515±.0125	+.6683	+.0057±.0096	0.59	0.42
	3, 7	38	Second (2)	1.2573±.0093	1.2010±.0082	+.6616	+.0563±.0072	7.77	4.69
			Diff. (2)−(1)	−.0999±.0122	−.1505±.0140	−.0067	+.0506±.0122	.....	.....
			Diff./$E_{diff.}$	8.21	10.7	.....	4.15	.....	.....
Pima Egyptian and Meade Upland, combined series	1, 3, 5, 7	77	First (1)	1.3808±.0107	1.3522±.0093	+.7687	+.0286±.0069	4.13	2.12
	1, 3, 5, 7	77	Second (2)	1.2780±.0075	1.2067±.0068	+.7222	+.0713±.0054	13.3	5.91
			Diff. (2)−(1)	−.1028±.0103	−.1455±.0112	−.0465	+.0427±.0082	.....	.....
			Diff./$E_{diff.}$	9.98	12.9	.....	5.22	.....	.....
Pima Egyptian and Acala Upland, from bulk seed	2, 4, 6	59	First (1)	1.3401±.0112	1.2998±.0106	+.6795	+.0403±.0088	4.60	3.10
	2, 4, 6	59	Second (2)	1.2804±.0076	1.1822±.0086	+.6441	+.0982±.0069	14.2	8.31
			Diff. (2)−(1)	−.0597±.0099	−.1176±.0139	−.0354	+.0579±.0095	.....	.....
			Diff./$E_{diff.}$	6.00	8.49	.....	6.11	.....	.....

values. For purposes of comparison it is desirable to reduce these to a percentage basis by dividing all of the absolute differences ($\times 100$) by the values for the Upland cotton.[14] The resulting percentage differences appear in column 10.

The second set of differences—those entered under the average constants for the first and second collections of Egyptian and Upland cottons, respectively, in columns 5 and 6, under the first and second correlation coefficients in column 7, and under the differences between Egyptian and Upland cotton in the first and second series in column 8—shows the relative values of the constants secured in the first and in the second series of determinations.

All of these differences are taken—

(constant for second series) *minus* (constant for first series).

All of the differences have been taken algebraically, with regard to the signs of the constants compared.

Since the physical constants for the two types of cotton (columns 5 and 6) are both positive values, a positive sign indicates a higher value of the physical constant in the second series. Since the correlations (column 7) and the differences between the two types of cotton (column 8) may theoretically be either positive or negative (and actually differ with regard to sign in some of the subsequent tables), it is necessary in interpreting these differences to bear in mind the signs of the constants for the first and second series between which comparisons are being drawn.

The ratios of these differences to their probable errors (when probable errors have been determined) are shown below the differences (opposite Diff./$E_{diff.}$).[15]

In determining the probable errors of differences between the means for the two series (columns 5 and 6) the correlation between the constants of the first and second series of determinations must be considered. The standard deviations of the differences are calculated from the formula

$$\sigma^2_{(2-1)} = \sigma_1^2 + \sigma_2^2 - 2r_{12}\sigma_1\sigma_2,$$

where the sigmas denote standard deviations and r the correlation coefficient measuring the relationship between the first and second series, as designated by the subscript numerals.[16]

The differences between the differences between Egyptian and Upland cotton in the first and second series (column 8, opposite "Diff., (2)−(1)") show whether the two types show greater or less differentiation at the time of the second than at the time of the first collection of samples.

The probable errors of the increase in the differences between the two kinds of cotton have been calculated as follows:

Let E. U. denote Egyptian and Upland plants, of the first or second series as may be indicated by subscript numerals. Then the differences are $E_1 - U_1$ and $E_2 - U_2$. The numerical value under consideration in

[14] In the case of hydrogen-ion concentration (Table XIII) the value of the deviation of the P_H for the Upland cotton from neutrality has been used in the calculation of the percentage difference between the two types.

[15] These values, like the ratios mentioned immediately above, have been computed from the original constants carried to a larger number of places than those given here, and are consequently slightly more accurate than those which may be recomputed from the constants as given in these tables.

[16] The coefficients of correlation, $[r]_{12}$, are given for each of the physicochemical constants considered, and for the different subcultures, in Table XXI. These coefficients will be considered later (p. 313). For the moment they are of interest merely as a means to the calculation of the probable errors of the differences between the constants for the first and second series of determinations.

the comparison of the first and second series is $(E_2-U_2)-(E_1-U_1)$. The means, standard deviations and correlations of these differences might be determined directly. Since the means and standard deviations of the individual determinations are both available and since the correlations between the individual determinations in the first and second series may be utilized for another investigation, it seems best to determine the correlations between the differences in the first series and the second series indirectly. The type of formula to be applied has been given elsewhere (*22*) and illustrated in its applicability to growth increments (*31*).

The formula to be used in the calculation of the probable error of the difference between the differences has also been given (*23*).

The standard deviations of the difference between the two parental forms have been determined in order to test the significance of the difference between the types in either the first or second series. We require, therefore, merely the correlations between the differences in the individual duplets or triplets, in the first series and in the second series.

In this case the product moment for the correlation between the differences, d_1, d_2

$$\Sigma (d_1d_2)=\Sigma (E_1E_2)-\Sigma (E_1U_2)-\Sigma (U_1E_2)+\Sigma (U_1U_2).$$

The standard deviation of the differences between the differences in the first and second series then becomes—

$$\sigma^2_{(d_2-d_1)}=\sigma_{d_1}{}^2+\sigma_{d_2}{}^2-2r_{d_1d_2}\sigma_{d_1}\sigma_{d_2}.$$

The correlations between these differences for the various characters appear in Table XXIII.

Turning now to a consideration of the actual constants in Table X we note first of all that the differences between Egyptian and Upland cotton (column 8) are without exception positive in sign. Thus they show that in all instances the leaf-tissue fluids of the Egyptian cotton have a higher osmotic concentration than those of the Upland. The differences in the first series are, in all cases but one, at least four times as large as their probable errors (column 9). The single exception occurs in the comparison between bulk Pima and bulk Meade. In the second series the differences are from 7.8 to 14.2 times as large as their probable errors. There can, therefore, be no question whatsoever concerning the significance of the differences between the osmotic concentration of the tissue fluids of Egyptian and Upland cotton. Expressing the results in terms of differences in atmospheres, P, rather than in freezing point lowering, Δ, by means of the formula

$$P=12.06\,\Delta-0.021\,\Delta^2,$$

for which tabled constants are available (*24*), it appears that the differences range from about 0.07 to 0.62 atmospheres in the first series. In the second series the differences range from 0.68 to 1.18 atmospheres.

The differences between the first and second series of determinations (Table X, columns 5 and 6) show that in the second series the osmotic concentration is regularly lower than that in the first series. These differences are in all cases six or more times as large as their probable errors. They show conclusively, therefore, that osmotic concentration is lower in the second series of determinations than in the first.

The correlations between members of the same duplet or triplet (column 7) are in all cases higher in the first than in the second series.

Since the problem of the magnitudes of the correlation between the members of the same duplet or triplet is not a subject for special discussion in the present analysis of the measurements, the probable errors of the differences between these correlation coefficients for the first and second series of determination have not been determined.

Finally, we note that the differences between Egyptian and Upland cotton are uniformly higher in the second than in the first series of collections. This is clearly shown by the absolute differences in column 8. The increase in the absolute difference between the Egyptian and Upland cotton from the first to the second series of determinations may be shown by taking the differences between these differences. These are shown immediately beneath the two differences.

The increase in the difference between Egyptian and Upland from the first to the second series of determinations is in all cases over three times as large as its probable error. In three cases it is over four times as large as its probable error.

Correlated with this larger difference we find a uniformly higher criterion of significance of difference (column 9) between the Egyptian and the Upland types in the second series.

The constants just discussed represent the absolute differences between the Egyptian and the Upland cottons as studied in the first and second series of determinations. It must be remembered, however, that in both Egyptian and Upland types the osmotic concentration is uniformly lower in the second series than in the first series of determinations. This means that the differences which have been shown to be absolutely larger in the second series than in the first are relatively much larger. This is clearly shown by the entries in the column (10) of Table X giving the percentage differences between the two types. In the first series of determinations, the relative differences range from less than 1 per cent to over 3.7 per cent. In the second series the percentage differences range from 4.7 per cent to 8.3 per cent.

The significance of these various comparisons will be more fully discussed in a subsequent section after the values of the other physical constants have been taken into account.

COMPARISON ON THE BASIS OF SPECIFIC ELECTRICAL CONDUCTIVITY

The statistical constants for the specific electrical conductivity of the tissue fluids of Egyptian and Upland cottons, expressed in terms of reciprocal ohms, $\kappa \times 10^6$, are compared in Table XI, which is made up in the same manner as Table X.

The results show that in every comparison (column 8) the tissue fluids of the Egyptian cotton have a higher specific electrical conductivity than those of the Upland varieties with which they are compared. A comparison of the differences with their probable errors (which have been calculated with due regard to the correlation obtaining between the constants of plants of the same duplet or triplet), shows (column 9) that in the first series all of the differences are over three times as large as their probable errors. In the second series all of the differences in electrical conductivity are over nine times as large as their probable errors.

TABLE XI.—*Comparison of specific electrical conductivity, $\kappa \times 10^6$, in Pima Egyptian and Meade and Acala Upland cotton, and in the first and second series of determinations for each type, in 1921*

(1) Types and varieties compared.	(2) Rows.	(3) N.	(4) Series or collection.	(5) Mean for Egyptian cotton.	(6) Mean for Upland cotton.	(7) Correlation between Egyptian and Upland.	Difference between Egyptian and Upland. (8) Absolute difference.	(9) Diff./$E_{diff.}$	(10) Percentage difference.
Pima Egyptian and Meade Upland, from self-fertilized seed.	1, 5	39	First (1).....	33,251±300	32,041±261	+.6753	+1,210±229	5.29	3.77
	1, 5	39	Second (2).....	30,643±237	28,056±222	+.3644	+2,587±259	9.99	9.22
			Diff. (2)−(1)...	−2,608±331	−3,985±356	−.3109	+1,377±314		
			Diff./$E_{diff.}$.....	7.87	11.2		4.38		
Pima Egyptian and Meade Upland, from bulk seed.	3, 7	38	First (1).....	32,074±291	31,141±253	+.5796	+933±251	3.71	2.99
	3, 7	38	Second (2).....	29,646±202	27,402±186	+.2844	+2,244±233	9.65	8.19
			Diff. (2)−(1)...	−2,428±339	−3,739±317	−.2952	+1,311±371		
			Diff./$E_{diff.}$.....	7.15	11.8		3.53		
Pima Egyptian and Meade Upland, combined series.	1, 3, 5, 7	77	First (1).....	32,671±216	31,597±185	+.6378	+1,074±173	6.21	3.40
	1, 3, 5, 7	77	Second (2).....	30,151±161	27,733±147	+.3584	+2,418±175	13.83	8.72
			Diff. (2)−(1)...	−2,520±230	−3,864±239	−.2794	+1,344±235		
			Diff./$E_{diff.}$.....	11.0	16.2		5.72		
Pima Egyptian and Acala Upland, from bulk seed.	2, 4, 6	59	First (1).....	32,028±216	31,117±181	+.4821	+911±204	4.47	2.93
	2, 4, 6	59	Second (2).....	29,981±169	27,606±158	+.4168	+2,375±177	13.42	8.60
			Diff. (2)−(1)...	−2,047±235	−3,511±256	−.0653	+1,464±275		
			Diff./$E_{diff.}$.....	8.70	13.7		5.32		

Thus it is clear that the Egyptian cotton contains larger quantities of conducting electrolytes in its leaf tissue fluids, just as it has been shown in the preceding section to contain larger quantities of osmotically active solutes.

Comparisons between the first and the second series show that in both Egyptian and Upland cotton (Table XI, columns 5 and 6) the electrical conductivity in the second series is invariably lower than in the first series. The differences are in all cases over 7.2 times as large as their probable errors and may be considered statistically significant in every instance.

The correlation between the plants of the same duplet or triplet is smaller in the second series than in the first. This result is in full agreement with that for osmotic concentration presented above. Since these correlations are not discussed in detail in this place, but merely served as a means of determining certain probable errors which are essential to the interpretation of the other constants, their probable errors, and the probable errors of the differences between them, have not been determined.

The differences between the two types of cotton (Table XI, column 8) are in all cases greater in the second than in the first series. The differences between Egyptian and Upland cotton in reciprocal ohms are over twice as great in the second as in the first series.

The ratio of these differences between the differences to their probable errors (calculated with due regard to the correlation between the differences themselves as explained above and shown in column 8) varies from 3.5 to 5.7 and may be reasonably considered to indicate statistically significant differences.

The ratios of the differences between the Egyptian and the Upland plants to their probable errors (column 9) are regularly larger in the second than in the first series of determinations.

Since the differences are based upon smaller absolute values in the second series than in the first, they are relatively larger than they appear to be as shown in column 8. The percentage differences in column 10 show that in the first series of determinations the Egyptian cotton has from 2.9 to 3.8 per cent higher electrical conductivity than the associated Upland cotton. In the second series of determinations, the Egyptian cotton is characterized by from 8.2 to 9.2 per cent higher electrical conductivity of its leaf tissue fluids.

COMPARISON ON THE BASIS OF THE RATIO OF SPECIFIC ELECTRICAL CONDUCTIVITY TO FREEZING POINT DEPRESSION

We now have to consider the relative proportion of electrolytes as indicated by the ratio of the specific electrical conductivity to freezing point lowering, κ/Δ.

The comparisons between the Upland and Egyptian varieties appear in Table XII which is constructed on the same principle as Table X. For the first series the differences between the Egyptian and the Upland types (column 8) are in three cases positive and one case negative in sign. In none of these four cases is the difference as much as two and one-half times as large as its probable error. We must, therefore, conclude that the two forms have practically the same average ratios.

TABLE XII.—*Comparison of ratio of specific electrical conductivity to freezing-point depression, $\kappa/\Delta 10^6$, in Pima Egyptian and Meade and Acala Upland cotton, and in the first and second series of determinations for each type, in 1921*

(1) Types and varieties compared.	(2) Rows.	(3) N.	(4) Series or collection.	(5) Mean for Egyptian cotton.	(6) Mean for Upland cotton.	(7) Correlation between Egyptian and Upland.	Difference between Egyptian and Upland. (8) Absolute Difference.	(9) Diff. / $E_{diff.}$	(10) Percentage difference.
Pima Egyptian and Meade Upland from self-fertilized seed.	1,5	39	First (1).....	23812±172	23753±141	+.4310	+59±169	0.35	0.25
	1,5	39	Second (2).....	23669±173	23195±151	+.1109	+474±217	2.18	2.04
			Diff. (2)−(1) ..	−143±194	−558±200	−.3201	+415±258		
			Diff./$E_{diff.}$.....	0.74	2.79		1.61		
Pima Egyptian and Meade Upland, from bulk seed.	3,7	38	First (1).....	23654±160	23139±213	+.4050	+515±208	2.47	2.23
	3,7	38	Second (2).....	23617±141	22864±168	+.5699	+753±145	5.19	3.29
			Diff. (2)−(1) ..	−37±171	−275±284	+.1649	+238±245		
			Diff./$E_{diff.}$.....	0.21	0.97		0.97		
Pima Egyptian and Meade Upland, combined series.	1,3,5,7	77	First (1).....	23734±118	23450±129	+.4064	+284±135	2.10	1.21
	1,3,5,7	77	Second (2).....	23643±112	23032±114	+.3189	+611±132	4.65	2.65
			Diff. (2)−(1) ..	−91±130	−418±173	−.0865	+327±184		
			Diff./$E_{diff.}$.....	0.70	2.42		1.78		
Pima Egyptian and Acala Upland, from bulk seed.	2,4,6	59	First (1).....	23969±119	24044±152	+.3826	−76±153	0.50	0.37
	2,4,6	59	Second (2).....	23451±113	23399± 93	+.4159	+52±113	0.46	0.22
			Diff. (2)−(1) ..	−517±145	−645±168	+.0333	+127±168		
			Diff./$E_{diff.}$.....	3.56	3.85		0.76		

In the second series the signs of all four of the differences (column 8) indicate a slightly higher ratio, κ/Δ, in the Egyptian plants. One of the differences is certainly insignificant in comparison with its probable error. The others may or may not be significant.

Finally, the percentage column (10) shows that the differences between Upland and Egyptian cotton do not exceed 3.3 per cent of the value of the constant for the Upland form.

The net result of this comparison seems to be that, taking the materials as a whole, there is no certain evidence of a difference in the relative values of κ in the two groups of cottons. Such differences as do appear are not wholly consistent from series to series and may be due merely to the errors of random sampling or to heterogeneity in the field upon which the plants were grown. The results for both the first and second series of determinations suggest, however, that the ratio is slightly higher in Egyptian than in Upland cotton. It will not be worth while to consider this problem further until conductivities can be corrected for the viscosity of the fluid, or until comparisons can be based on the actual analytical determination of one or more of the electrolyte ions.

A comparison of the ratio κ/Δ in the first and second series of determinations (Table XII) shows that in both Egyptian (column 5) and Upland (column 6) cotton, the ratio is somewhat smaller in the second than in the first series. These differences are not sufficiently large in comparison with their probable errors to be considered definitely significant as individual constants. The consistency of the results evidences strongly for a relatively lower concentration of electrolytes in the determinations made later in the season.

The differences between Egyptian and Upland cotton are apparently larger in the second than in the first series.

COMPARISON ON THE BASIS OF HYDROGEN-ION CONCENTRATION

While various authors have determined the total acidity of plant tissue fluids by titration, we have relatively little information on their actual acidity as determined by the systematic use of indicators, and fewer still by electrometric methods, giving the true hydrogen-ion concentration. Much of the work has been of a wholly incidental nature, and no attempt will be made here to review it in detail. Reference may be made to a recent summary by Atkins (*3*).

Haas (*18*, *19*), Truog (*50*), Hoagland (*34*), and Atkins (*1*) have shown that, in general, plant saps have a hydrogen-ion concentration well on the acid side of neutrality. Our findings for cotton are in agreement with this general experience, although the acidities which we have found are not so high as those stated by Atkins (*1*).

As far as we are aware, detailed studies of the relative values of hydrogen ion concentration in agricultural varieties have not been made heretofore.

The constants for the acidity of the tissue fluids of the Egyptian and Upland cottons measured in terms of P_H appear in Table XIII.

The average values of P_H in the various series of Egyptian cotton (column 5) range from 5.24 to 5.41, while in the Upland cotton (column 6) they range from 5.30 to 5.46. Thus all averages are well on the acid side of neutrality.

Comparing the Upland and Egyptian cotton by means of the difference column (8), we note that the P_H values for Egyptian cotton are in all cases lower than those for the Upland cottons.

The ratios of the differences to their probable errors (column 9) are in all cases larger than, and in some cases several times as large as, 3.5 times their probable error. There can, therefore, be no reasonable question concerning their significance. The results show clearly that the tissue fluids of the Egyptian cotton are distinctly more acid than those of the Upland cottons.

In the calculation of the percentage difference between the hydrogen-ion concentrations of the tissue fluids of the two species, a procedure must be adopted which is somewhat different from that used for the other constants. When expressed in terms of P_H, both acidity and alkalinity are measured from a neutral point determined by the dissociation constant of pure water. We may, therefore, logically take the deviation of the P_H value of the Upland cotton from neutrality as a base in calculating the percentage differences.

The exact value of P_H taken to represent neutrality is of relatively little importance. The dissociation constant of water varies with the temperature. We may arbitrarily take $P_H = 6.860$ for neutrality at the average temperature of our determinations. In determining the percentage difference between Egyptian and Upland cotton we therefore work from the formula

$$\frac{100\,(E-U)}{6.860-U}$$

where E and U represent the P_H values of Egyptian and Upland cottons, respectively.

The percentage differences (Table XIII, column 10) calculated in this way range from 2.75 to 8.67.

The correlations between the Upland and Egyptian plants of the same duplet or triplet (Table XIII, column 7) are of considerable interest. The values for the second series are very much larger than those for the first. All of the coefficients are positive in sign. These results are of importance in that they show that the hydrogen-ion concentrations of adjoining plants are correlated. This must be assumed to be due either to (*a*) extremely local differences in the soil of the field, or to (*b*) the influence of variations in the atmospheric conditions or in the time of collection of the samples. It is impossible at present to make any suggestions concerning the real factor to which these correlations are due. Their existence indicates clearly, however, that here is an important field for further investigation.

Turning now to the differences between the first and second collections (Table XIII, columns 5 and 6), we note that in all cases the value of P_H is somewhat larger in the second series than in the first. Thus the tissue fluids were on the average more nearly neutral at the time of the second series of determinations than when the first series was made.

A comparison of these differences with their probable errors shows that in every comparison except that based on plants grown from bulk seed of Meade in rows 3 and 7 the differences are at least 6 times as large as their probable errors.

There can, therefore, be no reasonable doubt concerning the validity of the conclusion that at the time of the taking of the second series of samples the tissue fluids of both Egyptian and Upland cultures were more nearly neutral than they were earlier in the season.

TABLE XIII.—*Comparison of hydrogen-ion concentration, P_H, in Pima Egyptian, Meade and Acala Upland cotton, and in the first and second series of determinations for each type, in 1921*

(1) Types and varieties compared.	(2) Rows.	(3) N.	(4) Series or collection.	(5) Mean for Egyptian cotton.	(6) Mean for Upland cotton.	(7) Correlation between Egyptian and Upland.	Difference between Egyptian and Upland. (8) Absolute difference.	(9) Diff. / $E_{diff.}$	(10) Percentage difference.
Pima Egyptian and Meade Upland, from self-fertilized seed.	1,5	39	First (1).....	5.245±.012	5.346±.012	+.4045	−.101±.013	7.84	6.67
	1,5	39	Second (2).....	5.335±.008	5.455±.009	+.5580	−.120±.008	14.7	8.54
			Diff.(2)−(1)....	+.090±.014	+.109±.014	+.1535	−.019±.015		
			Diff./E_{diff}	6.26	7.90		1.27		
Pima Egyptian and Meade Upland, from bulk seed.	3,7	38	First (1).....	5.269±.011	5.396±.012	+.1334	−.127±.015	8.48	8.67
	3,7	38	Second (2).....	5.378±.012	5.436±.012	+.6154	−.058±.010	5.54	4.07
			Diff.(2)−(1)....	+.109±.015	+.040±.018	+.4820	+.069±.019		
			Diff./E_{diff}	7.12	2.23		3.61		
Pima Egyptian and Meade Upland, combined series.	1,3,5,7	77	First (1).....	5.257±.008	5.370±.009	+.2949	−.113±.010	11.5	7.58
	1,3,5,7	77	Second (2).....	5.357±.007	5.446±.007	+.5530	−.089±.007	12.7	6.29
			Diff.(2)−(1)....	+.100±.011	+.076±.012	+.2581	+.024±.013		
			Diff./E_{diff}	9.45	6.46		1.94		
Pima Egyptian and Acala Upland, from bulk seed.	2,4,6	59	First (1).....	5.257±.009	5.302±.008	+.0475	−.045±.011	3.96	2.89
	2,4,6	59	Second (2).....	5.405±.008	5.444±.009	+.5271	−.039±.008	4.64	2.75
			Diff.(2)−(1)....	+.148±.011	+.142±.012	+.4796	+.006±.014		
			Diff./E_{diff}	13.4	11.8		0.46		

Comparing the differences between Egyptian and Upland cotton in the first and second series of determinations, we note that the differences between the differences (Table XIII, column 8) show that the second series has a numerically somewhat larger difference[17] in one of the three fundamental series, row 1 and 5, and a somewhat smaller difference in the other two, rows 3 and 7 and 2, 4 and 6. The difference between these differences can hardly be considered significant in comparison with their probable errors. The ratios range from 0.5 to 3.6.

It is clear, therefore, that the differentiation between the Egyptian and the Upland types with respect to the hydrogen-ion concentration of their tissue fluids can not be asserted to become greater with the advance of the season. This result for acidity, with respect to which differentiation has been shown to exist, is therefore in agreement with the ratio of specific electrical conductivity to freezing point depression, for which no differentiation has been demonstrated between the types. It is not in agreement with the results for freezing point depression, Δ, or specific electrical conductivity, κ, both of which showed an increase in the differences between the Egyptian and Upland cotton with the advance of the season.

In referring to the advance of the season, we must remember that there are but two groups of data upon which conclusions concerning seasonal changes may be based. Caution must, therefore, be used in attributing a seasonal significance to these results. The term is used here merely as a means of conveniently describing the actual facts.

PRESENTATION AND ANALYSIS OF DATA FOR HYBRIDS

The frequency distributions of the various physicochemical measurements made upon the hybrid in 1921 have been presented in tables VI–IX. For a more detailed comparison of the properties of the fluids of hybrid and parental forms we must resort to a consideration of the differences in the individual constants and in the statistical constants derived from these individual measurements.

The results for the preliminary measurements made in 1920 will first be given. The data of the more detailed studies made in 1921 will then be presented in the form of statistical constants.

PRELIMINARY STUDY IN 1920

We were able to secure the following determinations:

Acala × Pima F_1(29)[18] was taken on August 14. While not immediately associated with samples (1) = Egyptian and (2) = Upland, it may be most nearly compared with them. On August 18 Pima Egyptian × Upland F_1 (30) was taken between two rows of Pima (31). On the same date Pima Egyptian × Upland F_1 plants (32) were also taken in close proximity to Holdon Upland plants (33). On August 22 Pima Egyptian × Upland F_1 (34) was taken in comparison with Pima Egyptian (No. 25) and Holdon Upland (No. 26). In the same border we were so fortunate as to obtain a progeny of Acala × Pima F_1 (35) lying between

[17] Since the differences between the two types of cotton in column 8 are so taken as to have a negative sign when P_H is lower for Egyptian than for Upland cotton, a negative sign for the difference between the differences indicates that the difference is numerically larger in the second series of determinations.

[18] The numbers in parentheses are those of our samples of hybrid tissues. The number of the determination on the parent type is also given for the differences. The actual constants for these parent type numbers will be found in Table I.

the progenies of the two individual parents of the hybrid, Pima (27), and Acala (28).

The constants are set forth in table XIV.

TABLE XIV.—*Physicochemical constants for the leaf tissue fluids of the F_1 hybrid between Egyptian and Upland cotton grown in 1920, and differences between constants for the hybrid and those for the parent types*

Type and varieties compared.	Sample number.[a]	Depression of freezing point, Δ	Osmotic concentration in atmospheres, P.	Specific electrical conductivity, κ.	Ratio of conductivity to depression, κ/Δ.
F_1 hybrid, Acala Upland×Pima Egyptian	(29)	1.124	13.53	0.03316	0.03000
Difference between F_1 hybrid and Pima Egyptian	(29)-(1)	−0.268	−3.21	−.00564	+.00213
Difference between F_1 hybrid and Acala Upland	(29)-(2)	−0.094	−1.12	+.00233	+.00468
F_1 hybrid, Pima Egyptian×Upland	(30)	1.295	15.58	.03015	.02328
Pima Egyptian	(31)	1.365	16.42	.03162	.02317
Difference between F_1 hybrid and Pima Egyptian	(30)-(31)	−0.070	−0.84	−.00147	+.00011
F_1 hybrid, Pima Egyptian×Upland	(32)	1.496	17.99	.03132	.02093
Holdon Upland	(33)	1.468	17.65	.03222	.02195
Difference between F_1 hybrid and Holdon Upland	(32)-(33)	+0.028	+0.34	−.00090	−.00102
F_1 hybrid, Pima Egyptian×Upland	(34)	1.327	15.96	.05141	.03874
Difference between F_1 hybrid and Pima Egyptian	(34)-(25)	−0.020	−0.24	−.00433	−.00263
Difference between F_1 hybrid and Holdon Upland	(34)-(26)	+0.077	+0.92	−.00013	−.00249
F_1 hybrid, Acala Upland×Pima Egyptian	(35)	1.087	13.08	.02801	.02578
Difference between F_1 hybrid and Pima Egyptian	(35)-(27)	−0.166	−1.99	−.00408	+.00017
Difference between F_1 hybrid and Acala Upland	(35)-(28)	−0.026	−0.32	+.00007	+.00068

[a] Constants for parental forms not given in this table appear under these numbers in Table I.

These results show that in all four comparisons between Egyptian cotton and the hybrid between Egyptian and Upland cotton the osmotic concentration is higher in the leaf-tissue fluids of the Egyptian than in those of the hybrid plants. In all four comparisons, the specific electrical conductivity indicates that there is a higher concentration of conducting electrolytes in the tissue fluids of the Egyptian than in those of the hybrid plants. In three of the four comparisons the ratio κ/Δ is higher in the hybrid than in the Egyptian cotton.

The results of the comparisons between the hybrid and the Upland cottons are more irregular. Since both hybrid and Upland cottons seem to differ from Egyptian cotton in the same direction, it is only natural to find that there is not such a conspicuous difference between hybrid and Upland as between hybrid and Egyptian.

The results of this preliminary study can not be taken as conclusive. They are, however, suggestive, and fully justified the more intensive investigation undertaken in 1921.

INVESTIGATIONS IN 1921

COMPARISONS ON THE BASIS OF FREEZING-POINT DEPRESSION

A comparison of the freezing-point depressions of the tissue fluids of the hybrid with its two parent forms appears in Table XV.

This table (which may serve as a type for the others of this section) is prepared as follows: The forms compared with the hybrid are given

in column 1, the number of the row in the experimental field in column 2, the number of samples upon which the statistical constants are based in column 3, and the series of collections (first or second) in column 4. The average value of the freezing-point lowering for the hybrid form appears in column 5. The freezing-point lowering for the parent forms is given in column 6. The table is divided into two sections in order to distinguish between the Egyptian (upper half of table) and Upland (lower half of table) parents.

The correlation between the parents and the hybrids of the same triplet, given in column 7 of Table XV, is essential to the determination of the probable errors of the differences between the hybrid and the parent forms and will not be discussed in this place further than to note that the differences between the correlations for the first and second series show greater irregularities than those which were found in the coefficients for the two parental forms (p. 288).

Two sets of differences are given.

The first is that between the hybrid and parent forms (columns 8–10 of Table XV). The probable errors of these absolute differences have been calculated with due regard to the influence of the heterogeneity of the field and of the variations of time and atmospheric conditions upon the probable errors, i. e., by the use of the formula

$$\sigma^2_{(h-p)} = \sigma_h^2 + \sigma_p^2 - 2r_{hp}\sigma_h\sigma_p$$

where h = hybrid and p = parent forms, and the other symbols have their usual biometric significance. The ratios of the differences to their probable errors appear in column 9.[19]

The second set of differences comprises those between the means for the first and second series of determinations on hybrids and parents (columns 5 and 6 of Table XV), between the correlations between parents and hybrids (column 7) in the two series, and between the differences between the hybrid and the parent forms (column 8) in the first and second series, respectively. These differences are all taken with regard to signs. The ratio of these differences to their probable errors (when probable errors have been determined) are given beneath the differences.[20] The probable errors of the differences between the first and second collections for the hybrid and the parent forms (columns 5 and 6) have been computed with due regard to the correlation between the determinations for the first and second series, as set forth in Table XXI. The probable errors of the differences between the differences between hybrid and parent forms (column 8) have been computed with regard to the correlation between them (as given in Table XXIII) by means of a formula similar to that given in the last paragraph above.

Considering the results of the determinations (Table XV), it is clear that the differences between the hybrid and the parent form are negative in sign throughout, thus showing that the hybrid has in all comparisons a lower osmotic concentration of tissue fluids than either of the parental forms.

[19] These ratios have been computed from means and probable errors carried to a larger number of significant figures than it is possible to publish in these tables (Tables XV–XVIII). They are, therefore, somewhat more exact than those which may be deduced from the constants as given here.

[20] These ratios, like those referred to in the note immediately above, have been calculated from constants carried to a larger number of places.

TABLE XV.—*Comparison of freezing-point depression, Δ, in the F_1 hybrid with that in Pima Egyptian and Meade Upland Cotton, and in the first and second series of determinations for each type, in 1921*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	Difference between hybrid and parent.		
Type and variety of parent compared with hybrid.	Row.	N.	Series or collection.	Mean for hybrid.	Mean for parent.	Correlation between hybrid and parent.	(8) Absolute difference.	(9) Diff. / $E_{diff.}$	(10) Percentage difference.
Pima Egyptian from self-fertilized seed.	1,5	39	First (1).....	1.3004±.0148	1.4037±.0179	+.8407	−.1033±.0097	10.7	7.36
Do.....	1,5	39	Second (2).....	1.1373±.0105	1.2983±.0113	+.7324	−.1610±.0080	17.8	12.4
			Diff. (2)−(1)....	−.1631±.0175	−.1054±.0165	−.1083	−.0577±.0097		
			Diff./$E_{diff.}$.....	9.34	6.39		5.92		
Pima Egyptian from bulk seed.....	3,7	38	First (1).....	1.2928±.0094	1.3572±.0110	+.6002	−.0644±.0092	6.98	4.75
Do.....	3,7	38	Second (2).....	1.1027±.0100	1.2573±.0093	+.7188	−.1546±.0072	21.3	12.3
			Diff. (2)−(1)....	−.1901±.0135	−.0999±.0122	+.1186	−.0902±.0110		
			Diff./$E_{diff.}$.....	14.1	8.21		8.20		
Pima Egyptian combined series.....	1,3,5,7	77	First (1).....	1.2966±.0087	1.3808±.0107	+.7756	−.0842±.0068	12.4	7.00
Do.....	1,3,5,7	77	Second (2).....	1.1202±.0074	1.2780±.0075	+.7346	−.1578±.0054	29.1	12.3
			Diff. (2)−(1)....	−.1764±.0111	−.1028±.0103	−.0410	−.0736±.0074		
			Diff./$E_{diff.}$.....	15.9	9.98		10.0		
Meade Upland from self-fertilized seed.	1,5	39	First (1).....	1.3004±.0148	1.3528±.0137	+.7985	−.0524±.0091	5.77	3.87
Do.....	1,5	39	Second (2).....	1.1373±.0105	1.2122±.0107	+.8149	−.0749±.0065	11.6	6.18
			Diff. (2)−(1)....	−.1631±.0175	−.1406±.0175	+.0164	−.0225±.0101		
			Diff./$E_{diff.}$.....	9.34	8.06		2.21		
Meade Upland from bulk seed.....	3,7	38	First (1).....	1.2928±.0094	1.3515±.0125	+.6175	−.0587±.0099	5.90	4.34
Do.....	3,7	38	Second (2).....	1.1027±.0100	1.2010±.0082	+.6670	−.0983±.0076	12.9	8.18
			Diff. (2)−(1)....	−.1901±.0135	−.1505±.0140	+.0495	−.0396±.0112		
			Diff./$E_{diff.}$.....	14.1	10.7		3.54		
Meade Upland combined series.....	1,3,5,7	77	First (1).....	1.2966±.0087	1.3522±.0093	+.7291	−.0556±.0066	8.35	4.11
Do.....	1,3,5,7	77	Second (2).....	1.1202±.0074	1.2067±.0068	+.7477	−.0865±.0051	17.1	7.17
			Diff (2)−(1).....	−.1764±.0111	−.1455±.0112	+.0186	−.0309±.0075		
			Diff./$E_{diff.}$.....	15.9	12.9		4.12		

Since (1) the hybrid is not intermediate between the two parents with respect to the freezing point depression of its leaf tissue fluids but is characterized by a lower osmotic concentration than either of the parental forms, and since (2) the Egyptian parent is characterized by a higher osmotic concentration than the Upland parent, it is inevitable that the difference between the hybrid and the Egyptian parent should be far greater than that between the hybrid and the Upland parent. This is clearly evident in both the average value of the differences and in the ratio of these differences to their probable errors. The differences are, however, in all cases clearly significant in comparison with their probable errors. The ratios range from 5.8 to 29.1, and therefore leave no possible doubt as to the significance of the differences.

The differences between the first and second series of Egyptian and Upland cottons have been discussed above (p. 290). The comparisons of the constants for the first and second collection of hybrids (column 5 of Table XV) shows that in the heterozygous plants, as well as in the homozygous individuals of the parental strains, the second collection is characterized by a lower average osmotic concentration than the first. A remarkable fact in regard to these determinations is that in each of the six possible comparisons between the hybrid and the parental forms the hybrid shows greater decrease in osmotic concentration between the first and second collections than do either of the parent forms.[21]

A comparison of the differences between the hybrid and the parent forms in the first and second series, H_1–E_1, H_1–U_1, H_2–E_2, and H_2–U_2 by means of the differences between them (H_2–E_2)–(H_1–E_1), (H_2–U_2)–(H_1–U_1) is possible on the basis of the values in column 8 of Table XV. These show that in every instance the difference is larger in the second series than in the first.[22] Thus the differentiation between the parent and the hybrid individuals tends to become greater with the advance of the season, just as the differentiation of the Egyptian and Upland forms has been shown to become larger. This rule holds, notwithstanding the fact that the actual values of the osmotic concentration are in all cases (in both hybrid and parental individuals) lower in the second series than in the first.

Comparing the seasonal increases in the numerical magnitudes of the differences between parent and hybrid forms with their probable errors, calculated by the method indicated on page 290, we note ($Diff/E_{diff.}$ in column 8 of Table XV) that in general they may be considered statistically significant.

The percentage differences (in the final column of Table XV) have been calculated with the constant for the parental form as a base. In the comparisons between hybrid and Egyptian cotton, it appears that the hybrid has an osmotic concentration from 4.8 to 7.4 per cent lower than that of the Egyptian parent in the first series of determinations. In the second series of determinations the hybrid is characterized by an osmotic concentration of about 12 per cent lower than that of the

[21] A possible explanation of this difference may be found in the fact that there was but a single plant of the hybrid in each instance. It is possible that because of the necessity for a somewhat closer picking of leaves in the case of the single hybrid plant, the average age of the leaves may have been somewhat less in the second collection than in the case of the controls. This is, however, merely a suggested possibility. A solution of the problem must await further investigation.

[22] Since the differences between parent and hybrid forms are so taken that a negative sign indicates a lower value of freezing-point depression in the hybrid than in the parent, and since consistency in treatment throughout the paper demands the regarding of signs in taking the differences between the differences in the first and second series of determinations, a negative sign for the differences between the differences indicates that the differences between parent and hybrid are greater in the second series than in the first.

Egyptian plants growing beside it in the field. The hybrid has an osmotic concentration of its leaf-tissue fluids from 3.9 to 4.3 per cent lower than that of the Upland parent in the first series and from 6.2 to 8.2 per cent lower in the second series of determinations.

COMPARISON ON THE BASIS OF SPECIFIC ELECTRICAL CONDUCTIVITY

The comparison of the specific electrical conductivities of the tissue fluids of the hybrids with their two parent forms is made in Table XVI. which is constructed on the same plan as Table XV.

The results are uniform throughout in showing a lower specific electrical conductivity in the hybrid individual than in either the Egyptian or the Upland parent.

The differences between the hybrid and either the self-fertilized or the bulk Pima cultures are relatively large, the averages for the hybrid ranging from 0.001409 to 0.002700 reciprocal ohm [23] lower than those for the Pima controls. These differences are from 4.3 to 17.3 times as large as their probable errors. For the whole series (rows 1, 3, 5, and 7), the specific electrical conductivity of the hybrid is lower by 0.002016 reciprocal ohm in the first series and by 0.002690 reciprocal ohm in the second series. These differences are 9.9 and 17.3 times as large as their respective probable errors.

Since the Upland cottons are characterized by leaf-tissue fluids of lower specific electrical conductivity than those of Egyptian cottons (p. 291), the difference between the hybrid and Upland parent is necessarily much smaller than that between the hybrid and the Egyptian parent. While the differences in all cases indicate a lower electrical conductivity in the tissue fluids of the hybrid than in those of its Upland parent, they can not in general be considered significant in comparison with their probable errors.

The differences between the hybrid and the Pima parent (Table XVI, column 8) are greater[24] in the second series of determinations than in the first series. This is in agreement with the results for osmotic concentration of hybrid and parent forms discussed above. It is also in conformity with the results of a comparison of the differentiation of the parental types.

In the comparison between the hybrid and the Upland parent, where the differences are necessarily smaller, the differences between the hybrid and the parent form are smaller in the second series of determinations.

COMPARISON ON THE BASIS OF THE RATIO OF SPECIFIC ELECTRICAL CONDUCTIVITY TO FREEZING-POINT DEPRESSION

The comparisons are made in Table XVII.

In the first series of determinations the differences between hybrid and parent are in part positive and in part negative in sign. They are generally small, and can not be considered statistically significant in comparison with their probable errors.

[23] In the tables these are given in terms of reciprocal ohms$\times 10^5$.

[24] Note that since the differences between hybrid and parent form have negative signs, the law of signs requires that the differences between the differences have the negative sign when the differences are larger in the second series.

TABLE XVI.—*Comparison of specific electrical conductivity, $\kappa \times 10^6$, in the F_1 hybrid with that in Pima Egyptian and Meade Upland cotton, and in the first and second series of determinations for each type, in 1921*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	Difference between hybrid and parent.		
Type and variety of parent compared with hybrid.	Row.	N.	Series or collection.	Mean for hybrid.	Mean for parent.	Correlation between hybrid and parent.	(8) Absolute difference.	(9) $\frac{\text{Diff.}}{E_{diff.}}$	(10) Percentage difference.
Pima Egyptian from self-fertilized seed.	1, 5	39	First (1).....	30645±235	33251±300	+.6830	−2606±221	11.8	7.84
Do	1, 5	39	Second (2).....	27964±211	30643±237	+.5774	−2679±207	12.9	8.74
			Diff. (2)−(1).....	−2681±311	−2608±331	−.1056	−73±256		
			Diff./E_{diff}	8.61	7.87		0.29		
Pima Egyptian from bulk seed.....	3, 7	38	First (1).....	30666±231	32074±291	+.2247	−1408±328	4.30	4.39
Do.....	3, 7	38	Second (2).....	26946±250	29646±202	+.4141	−2700±232	11.6	9.11
			Diff. (2)−(1).....	−3720±303	−2428±339	+.1894	−1292±333		
			Diff./E_{diff}	12.2	7.15		3.88		
Pima Egyptian, combined series.....	1, 3, 5, 7	77	First (1).....	30655±164	32671±216	+.4490	−2016±205	9.85	6.17
Do.....	1, 3, 5, 7	77	Second (2).....	27461±159	30151±161	+.5281	−2690±155	17.3	8.92
			Diff. (2)−(1).....	−3194±221	−2520±230	+.0791	−674±217		
			Diff./E_{diff}	14.5	11.0		3.11		
Meade Upland from self-fertilized seed.	1, 5	39	First (1).....	30645±235	32041±261	+.5557	−1396±235	5.95	4.35
Do.....	1, 5	39	Second (2).....	27964±211	28056±222	+.3109	−92±254	0.36	0.33
			Diff. (2)−(1).....	−2681±311	−3985±355	−.2448	+1304±317		
			Diff./E_{diff}	8.61	11.2		4.11		
Meade Upland from bulk seed.....	3, 7	38	First (1).....	30666±231	31141±253	+.3179	−475±283	1.68	1.53
Do.....	3, 7	38	Second (2).....	26946±250	27402±186	+.1509	−456±269	1.69	1.66
			Diff. (2)−(1).....	−3720±303	−3739±317	−.1670	+19±399		
			Diff./E_{diff}	12.2	11.8		0.05		
Meade Upland, combined series.....	1, 3, 5, 7	77	First (1).....	30655±164	31597±185	+.4343	−942±187	5.04	2.98
Do.....	1, 3, 5, 7	77	Second (2).....	27461±159	27733±147	+.2671	−272±186	1.46	0.98
			Diff. (2)−(1).....	−3194±221	−3864±239	−.1672	+670±256		
			Diff. /E_{diff}	14.5	16.2		2.62		

In the second series of determinations, the differences are positive in all cases, and in all cases conspicuously larger than in the first series. They are from 4.7 to 13 times as large as their probable errors. There can be no reasonable doubt of the significance of these differences.

The increase in the difference between the hybrid and the parental form (either Upland or Egyptian) from the first to the second series of determinations is from 2.8 to 6.6 times as large as its probable error. (Diff./$E_{diff.}$ in column 8.) The difference between the hybrid and the parental form with respect to the ratio of specific electrical conductivity to freezing point lowering may therefore be confidently asserted to increase from the first to the second series of determinations.

A comparison of the first and second series of determinations with respect to the ratio of κ to Δ in the hybrids (Table XVII, column 5) and in the two cultures of the parent forms (column 6) shows that in the hybrid the ratio is higher in the second than in the first series of determinations. All of the differences are over three times as large as their probable errors, and may reasonably be regarded as statistically significant.

In the parent forms, both Egyptian and Upland, the ratio of conductivity to freezing-point depression is lower in the second than in the first series of determinations. Thus the parent forms are quite different from the hybrids in this regard. It must be noted, however, that the differences are small in the case of the parent plants, and can not be considered individually significant in comparison with their probable errors.

Expressed in relative terms (Table XVII, column 10) the differences show that in the first series of determinations the ratio is on the average from -0.01 to $+2.7$ per cent higher in the hybrid than in either the Egyptian or Upland parent form. In the second series the ratio is from 3.7 to 7.1 per cent higher in the heterozygous than in the homozygous plants.

The fact that the ratio in the hybrid increases from the first to the second series of determinations, whereas that in both parent forms decreases accounts for the higher percentage difference between the hybrid and each of its parent forms (column 10) in the second as compared with the first series.

COMPARISON ON THE BASIS OF HYDROGEN-ION CONCENTRATION

A comparison of the acidity of the leaf tissue fluids of the hybrid with that of the parent form is made in Table XVIII.

The difference column (column 8) shows at once that the hybrid is characterized by a larger value of P_H than is that of the Egyptian parental form. In the comparison between the hybrid and the Upland parent exactly the reverse is true; the hybrid has in each series a numerically smaller average value of P_H than has the Upland parent form.

The differences are in all but two cases over two and a half times as large as their probable errors. These figures and the general consistency of the results for the various series are strong evidences for the soundness of the conclusions drawn.

Thus the hybrid is intermediate in acidity between the two parental forms.

XVII.—*Comparison of ratio of specific electrical conductivity to freezing-point depression, $\kappa/\Delta \times 10^6$, in the F_1 hybrid with that in Pima Egyptian and Meade Upland cotton, and in the first and second series of determinations for each type, in 1921*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	Difference between hybrid and parent.		
Type and variety of parent compared with hybrid.	Row.	N.	Series or collection.	Mean for hybrid.	Mean for parent.	Correlation between hybrid and parent.	(8) Absolute difference.	(9) Diff. / $E_{diff.}$	(10) Percentage difference.
Pima Egyptian from self-fertilized seed	1, 5	39	First (1)	23706±208	23812±172	+0.4624	−106±199	0.53	0.45
Do	1, 5	39	Second (2)	24642±130	23669±173	+.1035	+973±206	4.73	4.11
			Diff. (2)–(1)	+936±244	−143±194	−.3589	+1079±273		
			Diff./$E_{diff.}$	3.83	0.74		3.95		
Pima Egyptian from bulk seed	3, 7	38	First (1)	23756±160	23654±160	−.0547	+102±232	0.44	0.44
Do	3, 7	38	Second (2)	24487±156	23617±141	+.5378	+870±140	6.22	3.68
			Diff. (2)–(1)	+731±236	−37±171	+.6125	+768±271		
			Diff./$E_{diff.}$	3.10	0.21		2.83		
Pima Egyptian combined series	1, 3, 5, 7	77	First (1)	23731±131	23734±118	+.2515	−3±153	0.02	0.01
Do	1, 3, 5, 7	77	Second (2)	24566±101	23643±112	+.3158	+922±125	7.37	3.90
			Diff. (2)–(1)	+835±170	−91±130	+.0643	+925±195		
			Diff./$E_{diff.}$	4.91	0.70		4.75		
Meade Upland from self-fertilized seed	1, 5	39	First (1)	23706±208	23753±141	+.3898	−47±200	0.24	0.20
Do	1, 5	39	Second (2)	24642±130	23195±151	−.1345	+1447±212	6.82	6.24
			Diff. (2)–(1)	+936±244	−558±200	−.5243	+1494±249		
			Diff./$E_{diff.}$	3.83	2.79		6.00		
Meade Upland from bulk seed	3, 7	38	First (1)	23756±160	23139±213	+.1692	+617±244	2.53	2.67
Do	3, 7	38	Second (2)	24487±156	22804±168	+.1675	+1623±209	7.76	7.10
			Diff. (2)–(1)	+731±2	−275±284	−.0017	+1006±343		
			Diff./$E_{diff.}$	3.10	0.97		2.93		
Meade Upland combined series	1, 3, 5, 7	77	First (1)	23731±131	23450±129	+.2522	+281±159	1.76	1.20
Do	1, 3, 5, 7	77	Second (2)	24566±101	23032±114	+.0405	+1534±118	13.0	6.67
			Diff. (2)–(1)	+835±170	−418±173	−.2117	+1253±191		
			Diff./$E_{diff.}$	4.91	2.42		6.56		

TABLE XVIII.—*Comparison of hydrogen-ion concentration, P_H, in the F_1 hybrid with that in Pima Egyptian and Meade Upland cotton, and in the first and second series of determinations for each type in 1921*

(1) Type and variety of parent compared with hybrid.	(2) Row.	(3) N.	(4) Series or collection.	(5) Mean for hybrid.	(6) Mean for parent.	(7) Correlation between hybrid and parent.	Difference between hybrid and parent. (8) Absolute difference.	(9) Diff./$E_{diff.}$	(10) Percentage difference.
Pima Egyptian from self-fertilized seed	1, 5	39	First (1)	5.316±.011	5.245±.012	+.2735	+.071±.014	5.22	4.40
Do	1, 5	39	Second (2)	5.392±.012	5.335±.008	+.6175	+.057±.010	5.79	3.74
			Diff. (2)–(1)	+.076±.018	+.090±.014	+.3440	−.014±.017		
			Diff./$E_{diff.}$	4.29	6.26		0.87		
Pima Egyptian from bulk seed	3, 7	38	First (1)	5.298±.011	5.269±.011	+.4594	+.029±.011	2.59	1.82
Do	3, 7	38	Second (2)	5.395±.013	5.378±.012	+.7333	+.017±.009	1.77	1.15
			Diff. (2)–(1)	+.097±.017	+.109±.015	+.2739	−.012±.015		
			Diff./$E_{diff.}$	5.59	7.12		0.80		
Pima Egyptian combined series	1, 3, 5, 7	77	First (1)	5.307±.008	5.257±.008	+.3449	+.050±.009	5.60	3.12
Do	1, 3, 5, 7	77	Second (2)	5.393±.009	5.357±.007	+.6598	+.036±.007	5.34	2.40
			Diff. (2)–(1)	+.086±.012	+.100±.011	+.3149	−.014±.011		
			Diff./$E_{diff.}$	6.95	9.45		1.21		
Meade upland from self-fertilized seed	1, 5	39	First (1)	5.316±.011	5.346±.012	+.1243	−.030±.015	1.96	1.98
Do	1, 5	39	Second (2)	5.392±.012	5.455±.009	+.3207	−.063±.013	4.91	4.48
			Diff. (2)–(1)	+.076±.018	+.109±.014	+.1964	−.033±.020		
			Diff./$E_{diff.}$	4.29	7.90		1.74		
Meade upland from bulk seed	3, 7	38	First (1)	5.298±.011	5.396±.012	+.2278	−.098±.014	6.86	6.69
Do	3, 7	38	Second (2)	5.395±.013	5.436±.012	+.8285	−.041±.007	5.77	2.88
			Diff. (2)–(1)	+.097±.017	+.040±.018	+.6007	+.057±.016		
			Diff./$E_{diff.}$	5.59	2.23		3.60		
Meade upland combined series	1, 3, 5, 7	77	First (1)	5.307±.008	5.370±.009	+.1500	−.063±.011	5.90	4.23
Do	1, 3, 5, 7	77	Second (2)	5.393±.009	5.446±.007	+.5932	−.053±.007	7.57	3.75
			Diff. (2)–(1)	+.086±.012	+.076±.012	+.4432	+.010±.013		
			Diff./$E_{diff.}$	6.95	6.37		0.82		

In the determination of the percentage differences between the P_H of the hybrid and that of the parent, we have considered that the proper base for the calculation of a relative difference is the deviation of the value of P_H from that which may be taken as indicating neutrality. Thus in the calculation of these relative differences we have used the formula

$$\frac{100\,(H-P)}{6.860-P},$$

where H and P represent the average P_H values for parent, P, and hybrid, H, respectively. This is in agreement with the practice in comparing the two parental types.

The percentage differences calculated in this way range from 1.1 to 6.7.

Turning to the comparison of the first and second sets of determinations on the hybrid plants, we note that in all cases the average value of P_H is higher in the second series than it is in the first. Thus the tissue fluids in the second series of determinations are more nearly neutral than in the first series. These values range from 4.29 to 6.95 times as large as their probable errors. They may be considered unquestionably significant.

Concerning this result we have no explanation to offer, Caution must be observed in interpreting it as a regular developmental change, since in an irrigated crop grown under the severe conditions of the Southwestern deserts many factors must vary from one series of determinations to another. An interpretation of the differences in acidity should be one of the purposes to be considered in the planning of any future investigations in this field.

Turning to the correlations (Table XVIII, column 7), we note that the coefficients measuring the relationship between the hydrogen-ion concentration of the hybrid and of the plants of the parental form growing beside it in the fields are in all cases positive in sign. This result substantiates the findings in the comparison of the two types of cotton (Table XIII, p. 297), which shows clearly that acidity is to some extent determined by the varying conditions of the soil or of the atmosphere at the time at which the samples were taken.

The second series of correlations is numerically larger than the first. This indicates either (*a*) that the plants have become more closely related to their substratum or (*b*) that their atmospheric environment differs more widely from time to time in the second series than in the first, or (*c*) that the determinations in the second series were made with a considerably higher order of precision than those of the first series.

These results indicate clearly the importance of further investigations along these lines.

Comparing the differences between the hybrid and the parent form in the first series with that in the second series (Table XVIII, column 8) we find that in the comparison with Egyptian cotton the differences are in all cases greater in the first than in the second series, but these differences can not be considered significant in relation to their probable errors. In the comparison with Upland cotton the differences are numerically greater in the first series for rows 3 and 7 and for rows 1, 3, 5, and 7, but numerically greater in the second series for rows 1 and 5.

DISCUSSION OF RESULTS

The data presented in the foregoing sections lead to definite conclusions as far as matters of fact concerning the physicochemical properties investigated are concerned. The consistency of results for the two years, the close concordance of the results for the various subseries in the comprehensive experiment of 1921, and the large size of the differences when considered in comparison with their probable errors, leave no possible doubt as to the existence of differences between the tissue fluids of the Egyptian and Upland cotton, and between the F_1 hybrid and the two parental types, with respect to the physicochemical properties investigated.

This represents a distinct advance in our working knowledge of the characteristics of a highly important economic plant. The biologist would, however, like to see some explanation of the demonstrated differences in terms of more fundamental biological phenomena. He would at least like to be sure that every possible biological source of error is eliminated.

We may, therefore, review again certain possible explanations of the differences which have been demonstrated.

REVIEW OF BIOLOGICAL SOURCES OF ERROR

The probable errors of random sampling have been so reduced by the number of data accumulated that the conclusions are not open to criticism on the grounds of the results being due to chance.

Differences between the plant forms due to differences in distribution over a field characterized by diversity in soil conditions have been fully eliminated, unless we assume that the forms are remarkably different in the depth of root penetration, and that the soil is sufficiently different at the various levels to influence the physicochemical properties in a different manner.

At present we have no adequate information on this point. It is a question to which we hope to give some attention later.

A possible source of error—and one which seems largely unavoidable during the stages of development preceding maturity—is to be found in the differences in the growth habit of the two groups (Egyptian and Upland) of cotton. This renders the collecting of wholly comparable leaves somewhat difficult, but the greatest care was exercised in this work. Ultimately it may be possible to refer the differences here demonstrated to some more fundamental physiological or morphological phenomenon involving stage of development, but at present we are unable to do so.

DIFFERENCES BETWEEN THE FIRST AND SECOND SERIES OF DETERMINATIONS

We have now to consider certain outstanding features of these series of constants.

Conspicuous among these is the fact that all the constants differ between the first and the second series. This fact may be established by the comparison of the actual differences in the eight fundamental tables of constants (Tables X to XIII and XV to XVIII) with their probable errors. For convenience of reference and to facilitate the comparison of the various

constants, these differences have been expressed as percentages of the average value of the first determination in Table XIX. In this table the percentages are calculated by using the actual value of the first constant as a base, except in the case of P_H, where the deviation of the first constant from neutrality, taken as 6.86, has been used.

A comparison between the first and second series is facilitated by determining averages for all the differences for the same type of plants (Egyptian, Upland, or hybrid). In determining such averages the percentage differences for the individual subcultures may be used, but some correction must be made (by weighting) for the differences in the size of the subcultures. The averages of the change from first to second series in Table XIX have been computed for the Egyptian and Upland plants by weighting the constants for rows 1 and 5 and for rows 3 and 7 by two and that for rows 2, 4, and 6 by three and taking 7 as N in the determinations of the average. The averages for the hybrids have been calculated without weighting.

TABLE XIX.—*Percentage difference in average value of constants for first and second series of determinations in Pima Egyptian, Meade and Acala Upland, and F_1 hybrid cotton, in 1921*

Type and variety of cotton and nature of seed planted.	Rows.	Freezing-point depression, Δ	Specific electrical conductivity, κ	Ratio of conductivity to depression, κ/Δ	Hydrogen-ion concentration, P_H
Pima Egyptian from self-fertilized seed	1, 5	−7.51	−7.84	−0.60	+3.51
Pima Egyptian from bulk seed	3, 7	−7.36	−7.57	−.16	+6.85
Pima Egyptian combined series	1, 3, 5, 7	−7.44	−7.71	−.38	+6.24
Pima Egyptian from bulk seed	2, 4, 6	−4.45	−6.39	−2.15	+9.23
Average		−6.16	−7.14	−1.14	+7.50
Meade Upland from self-fertilized seed	1, 5	−10.39	−12.44	−2.35	+7.20
Meade Upland from bulk seed	3, 7	−11.14	−13.01	−1.19	+2.73
Meade Upland combined series	1, 3, 5, 7	−10.76	−12.23	−1.78	+5.10
Acala Upland from bulk seed	2, 4, 6	−9.05	−11.28	−2.68	+9.05
Average		−10.03	−11.82	−2.16	+6.78
F_1 Hybrid between Egyptian and Upland	1, 5	−12.54	−8.75	+3.95	+4.92
Do	3, 7	−14.70	−12.13	+3.08	+6.21
Do	1, 3, 5, 7	−13.60	−10.42	+3.52	+5.34
Average		−13.63	−10.44	+3.51	+5.57

Both osmotic concentration as measured by the depression of the freezing point, Δ, and specific electrical conductivity in reciprocal ohms, κ, are lower in the second series than the first. The values of P_H are higher in the second than in the first series, but since higher values of P_H indicate lower concentrations of the hydrogen ion, it is clear that the concentration of the hydrogen ion is also lower in the second series than in the first. Thus the concentration of total solutes (molecules and ions), of all conducting solutes, and of hydrogen ions, is less in the second series than in the first.

The ratio of specific electrical conductivity to freezing-point depression is lower in the second series of determinations than in the first series for both Egyptian and Upland cottons, but the percentage differences are small. Just the reverse is found in the hybrid. The explanation of these differences between the hybrids and the parent forms must await the results of further investigations.

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Two possible explanations of the decrease in osmotic concentration, specific electrical conductivity, and hydrogen-ion concentration from the first to the second series will at once suggest themselves.

Soil moisture may have been greater or, due to the retardation of growth toward the end of the season, the requirements for soil moisture may have been less, in the second than in the first series. As a consequence of either of these conditions, the turgidity of the leaves may have been somewhat higher in the second series. Thus through the dilution effect of higher turgidity the measures of the concentration of solutes might be lower in the second series.

It is quite conceivable that the rapidly developing bolls made much higher demands upon the solutes of the leaf tissue fluids in the second period than in the first and that the lower values of both the osmotic concentration and specific electrical conductivity in the second period may have been due primarily to the withdrawal of solutes from the leaf by the developing fruits.

It is obviously impossible to determine with certainty which of these suggested hypotheses is the more logical, or what other explanation may be the true one, without special investigation. It is worth while to note, however, that the decrease in hydrogen-ion concentration can hardly be explained by the simple assumption of relatively higher turgidity in the second series of determinations. The organic acids are in general so weakly ionized that dilution has but little influence on the concentration of the hydrogen-ion. Since dilution is a highly improbable explanation of the change in the hydrogen-ion concentration, it can hardly be assumed to be the most probable explanation of changes in the value of Δ and κ.

Some light may perhaps be thrown upon the problem by considering in a comparative way the percentage difference between the first and second series.

A comparison of the percentage changes in Egyptian and Upland cotton as given in Table XIX shows that the decrease in the concentration of total solutes and of electrolytes from the first to the second series of determinations is greater in the Upland than in the Egyptian cotton. The average value of the change in freezing-point depression, Δ, is 6.16 per cent in Egyptian and 10.03 per cent in Upland cotton. The average change in specific electrical conductivity, κ, is 7.14 in Egyptian and 11.82 in Upland cotton. This result has an important bearing upon the suggested hypothesis that the decrease in osmotic concentration and electrical conductivity from the first to the second series of determinations is due to the rapid withdrawal of solutes from the leaves by the developing bolls. If the factor of the withdrawal of solutes by the developing bolls is important enough to account for the decrease in the concentrations of total solutes and electrolytes from the first to the second series, it might reasonably be expected to account for certain of the differences between the various kinds of plants. We have no quantitative measure of the relative number of developing bolls in the various series of plants in the first and second series, but the general impression derived from an inspection of the two types of cotton in the field is that at the time of the first series of determinations the Upland plants had relatively more well developed bolls than the Egyptian plants, while by the time the second series of determination was made, the slower-developing Egyptian cotton had produced a more nearly comparable crop of fruits.

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Now the actual results for percentage change in concentration from the first to the second series are exactly the opposite of what might be expected from the hypothesis and from the apparent facts with regard to the fruiting of the cottons. Since the Upland cotton was well into the fruiting condition when the first series of determinations was made, while the Egyptian cotton was just beginning to form bolls, one might on the assumptions made expect to find the greatest change in the sap properties of the Egyptian plants since it is in these plants that the greatest change in physiological state with respect to fruiting seems to have taken place. The results are just the reverse.

It is interesting in this connection to compare the results for the hybrid and the parent forms. The hybrid series show a greater percentage decrease in the value of Δ than either the Upland or the Egyptian plants, and there was presumably less change in the condition with respect to fruiting between the first and the second series than in the case of the Egyptian cotton.

THE RELATIVE MAGNITUDES OF THE DIFFERENCE BETWEEN THE TWO SPECIES IN THE FIRST AND SECOND SERIES OF DETERMINATIONS

In the discussion of the individual constants we have noted that the difference between the Egyptian and Upland type is generally larger in the second than in the first series. This is true for freezing-point depression, specific electrical conductivity, and generally for the ratio of specific electrical conductivity to freezing-point depression. The differences between the differences in the varieties in the first and second series may be considered significant in the case of the two direct measurements, but are not sufficiently large to be regarded as certainly trustworthy in the various subseries in the case of the ratio. The results for hydrogen-ion concentration are uncertain since the signs of the differences between the differences are not wholly consistent, and only one of the differences (that for rows 3 and 7) may be considered statistically significant in comparison with its probable error.

It is a significant fact that while the second series uniformly shows a larger differentiation of the Egyptian and the Upland types for both freezing-point depression and specific electrical conductivity, the actual magnitudes of the physical constants are lower for both freezing-point depression, Δ, and specific electrical conductivity, κ, in the second than in the first series. The ratio κ/Δ is on the average consistently lower in the second than in the first series, but the differences between the means are small and can not be considered significant individually in comparison with their probable errors. This is also true for the hydrogen-ion concentration.

For convenience of comparison, all of the percentage differences between Egytian and Upland cotton given in column 10 of tables X to XIII have been laid side by side in Table XX.

TABLE XX.—*Comparison of percentage difference between Pima Egyptian, Meade and Acala Upland cotton, in the first and second series of determinations and for various constants, in 1921*

Comparison.	Rows.	Series or collection.	Freezing-point depression, Δ.	Specific electrical conductivity, κ.	Ratio of conductivity to depression, κ/Δ.	Hydrogen-ion concentration P_H.
Pima Egyptian from self-fertilized seed and Meade Upland from self-fertilized seed.	1, 5 1, 5	First (1).... Second (2)..	+3.76 +7.10	+0.77 +9.22	+0.25 +2.04	−6.67 −8.54
		Diff. (2)−(1).	+3.34	+8.45	+1.79	−1.87
Pima Egyptian from bulk seed and Meade Upland from bulk seed.....	3, 7 3, 7	First (1).... Second (2)..	+0.42 +4.69	+2.99 +8.19	+2.23 +3.29	−8.67 −4.07
		Diff. (2)−(1).	+4.27	+5.20	+1.06	+4.60
Pima Egyptian and Meade Upland, combined series.....	1, 3, 5, 7 1, 3, 5, 7	First (1).... Second (2)..	+2.12 +5.91	+3.40 +8.72	+1.21 +2.65	−7.58 −6.39
		Diff. (2)−(1).	+3.79	+5.32	+1.44	+1.29
Pima Egyptian and Acala Upland from bulk seed.....	2, 4, 6 2, 4, 6	First (1).... Second (2)..	+3.10 +8.31	+2.93 +8.60	−0.37 +0.22	−2.89 −2.75
		Diff. (2)−(1).	+5.22	+5.67	+0.59	+0.14

The reasons for the larger differences in the values of freezing-point lowering and specific electrical conductivity in the second as compared with the first series are difficult to determine. Among the possibilities may be mentioned:

(1) That the greater exactness of the constants in the second series, resulting from all the collections of samples having been made by one observer, has resulted in more definite differences in the two series.

(2) That the differentiation here under consideration is dependent upon the physiological age of the organism.

These are questions which await further investigation.

THE CORRELATION BETWEEN THE FIRST AND THE SECOND SERIES OF DETERMINATIONS

In the preceding discussion we compared the constants for the first and second series of measurements. To determine the probable errors of the differences between the two series, it was necessary to compute the coefficients of correlation between them by considering the homologous samples of the first and second series as the two variables of a pair and the number of homologous pairs as N.

The coefficients measuring the correlation between the determinations of the first and second series appear in Table XXI. Here the varieties and the rows are arranged as in preceding tables. The headings for Δ, κ, κ/Δ, and P_H indicate the correlations between the first and second series for the values of these constants. The table is broken into three sections, the upper for the Pima Egyptian, the second for the Upland, and the lower section for the hybrid.

The correlations between the first and second series of constants must be considered from two sides—first, that of the relative magnitude of the coefficients in the two species and their hybrid; second, that of the relative magnitude of the correlations between the first and the second series of collections for the four physicochemical constants under consideration.

TABLE XXI.—*Comparison of correlation between the first and second series of determinations for Egyptian and Upland cotton and their F_1 hybrid, in 1921*

Type, variety, and nature of seed planted.	Rows.	Correlation for depression of freezing point, Δ.		Correlation for specific electrical conductivity, κ.		Correlation for ratio of conductivity to depression, κ/Δ.		Correlation for hydrogen-ion concentration, P_H.	
		$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r
Egyptian cotton:									
Pima, from selfed seed	1, 5	+.4350±.0876	4.97	+.2553±.1009	2.53	+.3681±.0934	3.94	−.0105±.1080	0.10
Pima, from bulk seed	3, 7	+.2863±.1005	2.85	+.2461±.1027	2.40	+.3586±.0953	3.76	+.0972±.1084	0.90
Pima, combined series	1, 3, 5, 7	+.4049±.0643	6.30	+.2854±.0706	4.04	+.3637±.0667	5.45	+.0726±.0765	0.95
Pima, from bulk seed	2, 4, 6	+.4939±.0664	7.44	+.2703±.0813	3.32	+.2143±.0837	2.56	+.1419±.0860	1.65
Average		+.4178	.....	+.2591	.....	+.2995	.....	+.0856	.....
Upland cotton:									
Meade, from selfed seed	1, 5	−.0070±.1080	0.06	−.0785±.1073	0.73	−.0665±.1075	0.62	+.1358±.1060	1.28
Meade, from bulk seed	3, 7	+.1247±.1077	1.16	−.0219±.1093	0.20	−.0992±.1083	0.92	−.1869±.1056	1.77
Meade, combined series	1, 3, 5, 7	+.0456±.0767	0.59	−.0200±.0768	0.26	−.0136±.0769	0.18	−.0627±.0766	0.82
Acala, from bulk seed	2, 4, 6	−.0233±.0878	0.27	−.1364±.0861	1.58	−.1299±.0863	1.51	−.0179±.0879	0.20
Average		+.0236	.....	−.0871	.....	−.1030	.....	−.0223	.....
Hybrid cotton:									
F_1, Pima×Meade	1, 5	+.0745±.1074	0.69	+.0276±.1079	0.26	+.0058±.1080	0.05	−.1371±.1060	1.29
F_1, Pima×Meade	3, 7	+.0306±.1093	0.28	+.1114±.1080	1.03	−.1169±.1079	1.08	−.0647±.1090	0.59
F_1, Pima×Meade	1, 3, 5, 7	+.0629±.0766	0.82	+.0672±.0765	0.88	−.0509±.0767	0.66	−.1019±.0761	1.34
Average		+.0525	.....	+.0695	.....	−.0556	.....	−.1009	.....

A casual glance at Table XXI will show that the Egyptian series on the one hand and the Upland and the hybrid series on the other differ materially with respect to the correlations between the first and second series of determinations. All of the coefficients for Δ, κ, and κ/Δ in the Pima series are positive and in general may be considered significant in comparison with their probable errors. The comparable coefficients for the Upland series and for the hybrid plants are characterized by low values of the correlation coefficients. These may be either positive or negative in sign. They are of the same general order of magnitude as their probable errors. No one of the 21 coefficients can be considered certainly significant in comparison with its probable error.

The coefficients for hydrogen-ion concentration are low throughout, and can not be considered statistically significant in comparison with their probable errors.

Taking the averages[25] of the constants for the parental groups and for the hybrid groups, we note that all of the coefficients for the Upland plants are numerically less than 0.11. One of them is positive while three are negative in sign. The averages for Δ, κ, and κ/Δ for the hybrid plants are less than 0.07. The average value of the two coefficients for hydrogen-ion concentration in the hybrid plants is -0.10.

Contrasted with these, we find in the Pima Egyptian series the following averages: $r = +0.418$ for Δ, $r = +0.259$ for κ, $r = +0.299$ for κ/Δ, and $r = +0.086$ for P_H. Clearly these correlations for the Egyptian series are far higher than those for the Upland series.

We have considered various possible explanations of this difference in correlation, but since actual data are wanting for the adequate substantiation or refutation of any of the suggested possibilities, it seems wisest to omit all discussions of suggested explanations until further experimental evidence, now being collected, can be analyzed.

That the behavior of the two types of plants is quite different is further suggested by a consideration of the cross correlations between the constants of the plants of the same duplet or triplet in the first and second series, as set forth in Table XXII. The coefficients in this table were determined primarily as a means of obtaining the probable errors of the differences between the first and second series of differences between the Egyptian and the Upland types. The few words of discussion are purely incidental. The problem will, we hope, receive more detailed treatment on the basis of further data now being collected.

The coefficients presented show the relationship between the first determination on the Egyptian plants of a duplet or triplet and the second determination on the Upland or hybrid plants of the same duplet or triplet; or, conversely, the constants show the correlation between the first determination on the Upland or hybrid plants of a duplet or triplet and the second determination on the Pima plants; or, finally, the coefficients show the relationship between the first determination on the Upland plants of a triplet and the second determination on the hybrid plants, or the first determination on the hybrid plants and the second determination on the Upland plants.

The first two columns of Table XXII show the plants which furnished the materials for the first and second determinations, respectively. For convenience of comparison these are arranged in pairs. The first

[25] The averages of the correlations have been computed for the Egyptian and the Upland plants by weighting the constants for rows 1 and 5 and for 3 and 7 by two and that for rows 2, 4 and 6 by three, and taking 7 as N in the determination of the average for the whole experiment.

TABLE XXII.—*Comparison of correlation between (a) the physicochemical constants for the first series of determinations based on Upland Cotton (Meade and Acala varieties) and on the F_1 hybrid and those of the second series of determinations based on Egyptian cotton (Pima variety) of the same duplet or triplet; and (b) the constants for the first series of determinations based on Egyptian cotton and those of the second series of determinations based on Upland cotton or on the F_1 hybrid of the same duplet or triplet, in 1921*

First series of determinations.	Second series of determinations.	Rows.	Correlations for depression of freezing point, Δ.		Correlations for specific electrical conductivity, κ.		Correlation for ratio of conductivity to depression, κΔ.		Correlation for hydrogen-ion concentration, P_H.	
			$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r
Meade Upland [a]	Pima Egyptian	1, 5	+.3176±.0971	3.27	−.1528±.1055	1.44	+.2707±.1001	2.70	+.0627±.1076	0.58
Pima Egyptian	Meade Upland	1, 5	+.1322±.1061	1.24	+.1913±.1040	1.83	+.0451±.1078	0.41	+.0283±.1080	0.26
	Difference		+.1854±.1438	1.28	−.3441±.1481	2.32	+.2256±.1471	1.53	+.0344±.1525	0.22
Meade Upland [b]	Pima Egyptian	3, 7	+.2650±.1017	2.60	+.2331±.1035	2.25	+.0972±.1083	0.89	−.0264± 1093	0.24
Pima Egyptian	Meade Upland	3, 7	+.1405±.1073	1.30	+.2185±.1042	2.09	+.1470±.1071	1.37	+.0655±.1090	0.60
	Difference		+.1245±.1478	0.84	+.0146±.1468	0.10	−.0498±.1523	0.33	−.0919±.1544	0.59
Acala Upland [c]	Pima Egyptian	2, 4, 6	+.3461±.0773	4.47	+.2038±.0842	2.42	+.0215±.0878	0.24	+.0745±.0873	0.85
Pima Egyptian	Acala Upland	2, 4, 6	−.0558±.0875	0.63	−.0555±.0875	0.63	+.0446±.0876	0.50	+.0434±.0876	0.49
	Difference		+.4019±.1168	3.44	+.2593±.1214	2.13	−.0231±.1240	0.18	+.0311±.1237	0.25
F_1 hybrid [a]	Pima Egyptian	1, 5	+.4049± 0903	4.48	+.1538±.1054	1.45	+.2937±.0986	2.97	−.0329±.1079	0.30
Pima Egyptian	F_1 hybrid	1, 5	+.0001±.1080	0.00	−.0366±.1079	0.34	−.1405±.1059	1.32	−.1440±.1058	1.36
	Difference		+.4048±.1408	2.87	+.1904±.1508	1.26	+.4342±.1446	3.00	+.1111±.1511	0.73
F_1 hybrid [b]	Pima Egyptian	3, 7	+.2344± 1034	2.26	+.0852±.1086	0.78	+.0197±.1093	0.18	−.0732±.1088	0.67
Pima Egyptian	F_1 hybrid	3, 7	+.0399±.1093	0.27	−.1326±.1075	1.23	+.2843±.1006	2.82	+.1972± 1052	1.87
	Difference		+.2045±.1505	1.35	+.2178±.1528	1.43	−.2646±.1485	1.78	−.2704±.1513	1.78
Meade Upland [a]	F_1 hybrid	1, 5	−.1093±.1067	1.02	−.3770±.0927	4.06	−.0825±.1073	0.76	+.0354± 1079	0.33
F_1 hybrid	Meade Upland	1, 5	+.0983±.1070	0.91	+.1532±.1055	1.45	−.2648±.1004	2.63	−.1867±.1042	1.79
	Difference		−.2076±.1511	1.37	−.5302±.1404	3.77	+.1823±.1469	1.24	+.2221±.1500	1.48
Meade Upland [b]	F_1 hybrid	3, 7	+.0921±.1085	0.84	−.0215± 1094	0.19	−.0224±.1079	0.20	−.1590±.1067	1.49
F_1 hybrid	Meade Upland	3, 7	+.1454±.1071	1.35	+.1497±.1070	1.39	+.0587±.1076	0.54	−.1282±.1076	1.19
	Difference		−.0533±.1525	0.34	−.1712±.1530	1.12	−.0811±.1523	0.53	−.0308±.1515	0.20

[a] Pima Egyptian and Meade Upland, both from self-fertilized seed.
[b] Pima Egyptian and Meade Upland, both from bulk seed.
[c] Pima Egyptian and Acala Upland, both from bulk seed.

two horizontal rows of constants, for example, show the correlations between the first collection of Meade and the second collection of Pima and the correlation between the first collection of Pima and the second collection of Meade for the same triplet.

To bring out clearly the relationships shown by these correlations it is necessary to determine the differences between the two coefficients of a pair. In determining and interpreting these differences we must bear in mind the fact that the mathematical coefficient of correlation may be either positive or negative in sign. Positive coefficients indicate that in the long run the two variables tend to deviate from the averages of their respective means in the same direction. By higher coefficient, as used in the comparison of the two coefficients of a pair in the present connection, we understand that which is numerically larger when both have the same sign, or the one which most nearly approached +1 when the sign of one or both of the coefficients is negative.

The differences in Table XXII are so taken that a positive sign indicates that the correlation is higher when (*a*) the Egyptian determination is made in the second series and the Upland determinations made in the first series, and (*b*) when the Egyptian determination is made in the second series and the hybrid determination in the first series, and (*c*) when the hybrid determination is in the second series and the Upland determination in the first series.

Limiting our attention for the moment to the cross correlations between the two parent types (i. e., omitting the correlations in which one constant is based upon a hybrid individual), we note that the coefficients are preponderantly positive in sign, thus showing that when a first determination on one variety is compared with a second determination made at a later date on a sample of tissue of another variety drawn from the same part of the plot there will be a measurable similarity between them. Individually the constants are low and can not in general be considered significant in comparison with their probable errors.

The correlations in which one of the two variables is a constant based on a hybrid plant are very irregular and can not in general be considered significant in comparison with their probable errors.

A comparison between the correlations for the two parent types shows that in 8 of the 12 comparisons the correlations are somewhat higher when the Upland culture furnished the first sample and the Egyptian culture furnished the second sample than when the reverse is true.

We have at present no explanation to offer for this difference in the behavior of the two types of plants. The results are set forth merely as matters of fact ascertained in the determination of correlations to be used primarily in the determination of probable errors. (See p. 289.) The explanation of the facts must await further research.

The correlations between the differences between the biological forms compared in the first series of determinations and the differences between the same forms as compared in the second series of determinations are given in Table XXIII. These are presented because they have been used in the determination of the probable errors of the differences between the differences.

The second problem, that of the relative magnitude of the coefficients for the four physicochemical measurements, will be considered when the problem of the magnitudes of the correlations for the same duplet or triplet is discussed. (See p. 319.)

TABLE XXIII.—*Comparison of correlation between the differences between the various types of plants (Pima Egyptian, Meade and Acala Upland and F_1 hybrid cotton) in the first and second series of determinations, in 1921*

Types and varieties between which differences have been taken in the first and second series of determinations.	Rows.	Correlation for depression of freezing point, Δ.		Correlation for specific electrical conductivity, κ.		Correlation for ratio of conductivity to depression, κ/Δ.		Correlation for hydrogen-ion concentration, P_H.	
		$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r
Pima Egyptian, from self-fertilized seed, and Meade Upland, from self-fertilized seed	1, 5	+.1773±.1046	1.69	+.1745±.1047	1.67	+.1258±.1063	1.18	+.0405±.1078	0.37
Pima Egyptian, from bulk seed, and Meade Upland, from bulk seed	3, 7	−.0201±.1094	0.18	−.1763±.1060	1.66	−.0779±.1088	0.72	−.1125±.1080	1.04
Pima Egyptian, from bulk seed, and Acala Upland, from bulk seed	2, 4, 6	+.2846±.0807	3.52	+.0449±.0876	0.51	+.2203±.0835	2.64	+.0073±.0878	0.08
Pima Egyptian, combined series, and Meade Upland, combined series	1, 3, 5, 7	+.1306±.0756	1.72	+.0093±.0769	0.12	+.0450±.0767	0.59	−.0801±.0764	1.04
Pima Egyptian, from self-fertilized seed, and F_1 hybrid	1, 5	+.4117±.0897	4.58	+.2897±.0989	2.93	+.0905±.1071	0.85	+.0326±.1079	0.30
Pima Egyptian, from bulk seed, and F_1 hybrid	3, 7	+.1231±.1078	1.14	+.3297±.0975	3.38	−.0073±.1094	0.07	−.1322±.1075	1.22
Pima Egyptian, combined series, and F_1 hybrid, combined series	1, 3, 5, 7	+.2881±.0705	4.08	+.2983±.0700	4.26	+.0182±.0766	0.24	+.0063±.0769	0.08
Meade Upland, from self-fertilized seed, and F_1 hybrid	1, 5	+.1816±.1044	1.73	+.1615±.1052	1.54	+.2679±.1002	2.67	+.0469±.1078	0.43
Meade Upland, from bulk seed, and F_1 hybrid	3, 7	+.2095±.1046	2.00	−.0410±.1092	0.38	−.1429±.1072	1.33	+.0543±.1091	0.49
Meade Upland, combined series, and F_1 hybrid, combined series	1, 3, 5, 7	+.2026±.0737	2.74	+.0549±.0766	0.72	+.0704±.0765	0.92	+.0189±.0768	0.24

THE CORRELATION BETWEEN THE MEMBERS OF THE SAME DUPLET OR TRIPLET

A casual examination of the fundamental Tables X to XIII and XV to XVIII will have shown that there is a material correlation between the sap properties of the plants of the same duplet or triplet. This correlation may, in part, be assumed to be due to substratum heterogeneity and in part to the differentiation of the individual duplets or triplets from the population as a whole by meteorological and time factors.

Further evidences of the existence of such differentiating factors have been furnished by the correlations between the first and second determinations on plants of the same duplet or triplet as set forth in Tables XXI and XXII.

It might be assumed that the coefficients of correlation between the physicochemical constants of the plants of the same duplet or triplet will furnish some measure of the susceptibility of these physicochemical constants (Δ, κ, κ/Δ, and P_H) to the influence of (*a*) the relatively permanent differentiation of the substratum and (*b*) the transient differentiation, due to variations in water content and atmospheric conditions. The closest correlations would be expected for the characters which are more susceptible to external influences. This assumption will be valid if all of the physicochemical properties have been measured with an approximately equal degree of accuracy, so that the errors of measurement will not materially influence the correlation coefficients.

We have, therefore, prepared a comparison of the correlations between the determinations of the same duplet or triplet in the various cultures. This comparison is made in Table XXIV. The constants given are the differences between the correlation coefficients as indicated in the headings of the columns. Thus $\Delta-\kappa$ indicates the difference between the correlation for freezing-point lowering and the correlation for specific electrical conductivity between the members of the duplets or triplets of the rows indicated. The original correlation coefficients are given in the eight fundamental tables X to XIII and XV to XVIII of constants.

The probable errors of the differences in the correlations between the various constants have not been determined, since we are not sure in how far the ordinary statistical theory of the probable error of the difference between two constants is applicable to the present case.

We have first to consider the correlation between the osmotic concentration of the plants of the same duplet or triplet in comparison with the magnitudes of the comparable coefficients for the other physicochemical properties.

The correlation coefficient for osmotic concentration, Δ, is in every instance higher than that for specific electrical conductivity, κ, and in every instance higher than that for the ratio of specific electrical conductivity to freezing-point lowering, κ/Δ. It is in nearly every case higher than that for hydrogen-ion concentration expressed in terms of P_H.

TABLE XXIV.—*Comparison of the correlations for the same duplet or triplet in 1921, for the various physico-chemical properties, the values being the differences between the coefficients of correlation for each pair of characters*

Member of duplet or triplet between which the correlation is determined.	Rows.	Difference between correlation for depression of freezing point, Δ, and specific electrical conductivity, κ. Δ–κ	Difference between correlation for depression of freezing point, Δ, and ratio of conductivity to depression, κ/Δ. Δ–κ/Δ	Difference between correlation for depression of freezing point, Δ, and hydrogen-ion concentration, P_H. Δ–P_H	Difference between correlation for specific electrical conductivity, κ, and ratio of conductivity to depression κ/Δ. κ–κ/Δ	Difference between correlation for specific electrical conductivity, κ, and hydrogen-ion concentration, P_H. κ–P_H	Difference between correlation for ratio of conductivity to depression, κ/Δ, and hydrogen-ion concentration P_H. κ/Δ–P_H
First series:							
Pima and Meade	1, 5	+0.1899	+0.4273	+0.4538	+0.2443	+0.2708	+0.0265
Do	3, 7	+.0887	+.2633	+.5349	+.1746	+.4462	+.2716
Do	1, 3, 5, 7	+.1309	+.3624	+.4738	+.2315	+.3430	+.1115
Pima and Acala	2, 4, 6	+.1974	+.2969	+.6320	+.0995	+.4346	+.3351
Hybrid and Pima	1, 5	+.1577	+.3783	+.5672	+.2206	+.4095	+.1889
Do	3, 7	+.3755	+.6550	+.1408	+.2794	−.2347	−.5142
Do	1, 3, 5, 7	+.3266	+.5241	+.4307	+.1975	+.1041	−.0934
Hybrid and Meade	1, 5	+.2428	+.4087	+.6741	+.1659	+.4314	+.2655
Do	3, 7	+.2996	+.4483	+.3896	+.1487	+.0901	−.0586
Do	1, 3, 5, 7	+.2948	+.4768	+.5790	+.1821	+.2843	+.1023
Second series:							
Pima and Meade	1, 5	+.4010	+.6545	+.2074	+.2535	−.1936	−.4470
Do	3, 7	+.3772	+.0917	+.0462	−.2855	−.3310	−.0455
Do	1, 3, 5, 7	+.3638	+.4033	+.1692	+.0395	−.1946	−.2341
Pima and Acala	2, 4, 6	+.2273	+.2282	+.1170	+.0009	−.1103	−.1112
Hybrid and Pima	1, 5	+.1530	+.6289	+.1149	+.4738	−.0401	−.5139
Do	3, 7	+.3047	+.1610	−.0145	−.1437	−.3193	−.1755
Do	1, 3, 5, 7	+.2004	+.4187	+.0748	+.2123	−.1316	−.3439
Hybrid and Meade	1, 5	+.5040	+.9494	+.4942	+.4554	−.0098	−.4552
Do	3, 7	+.5162	+.4995	−.1615	−.0166	−.6776	−.6610
Do	1, 3, 5, 7	+.4866	+.7072	+.1545	+.2265	−.3362	−.5527

For further evidence on this point we may turn to Table XXI, showing the correlation between the various constants in the first and second series.[26] The coefficients for the Upland and hybrid series have been shown to be so nearly zero that they may be disregarded for the moment. Considering the more substantial values of the correlation for the Egyptian cotton, it appears that the highest values found are those for Δ, which are of the order 0.40. The correlations for κ and κ/Δ are of the order 0.30. The correlations for P_H are sensibly zero.

These results seem to indicate that the absorption of electrolytes from the soil is not the most important, or at least the sole, factor in the differentiation of the plants. This is shown by the fact that the correlations for osmotic concentration are higher than those for electrical conductivity and for ratio of conductivity to freezing-point lowering. That synthesized solutes are of importance in differentiating the plants of the same duplet or triplet at the moment at which the collection is made, is shown by the substantial values of the correlations for hydrogen-ion concentration between the members of the same duplet or triplet, as demonstrated for the Egyptian and Upland plants in the seventh column of Table XIII and for the parental and the hybrid types in the seventh column of Table XVIII.

It is perhaps not surprising that the correlation for total solutes should be higher than that for either of its measured constituents.

[26] In view of the fact that we hope later to discuss in detail the problem of the relationship between the properties of the soil solution and those of the plant tissue fluids, it seems undesirable to consider the cross correlations between the first and second series of constants as set forth in Table XXII.

We may turn again to Table XXIV for a comparison of the magnitudes of the correlations for specific electrical conductivity, ratio of conductivity to freezing-point depression, and hydrogen-ion concentration.

In the first series the correlation for specific electrical conductivity is in every instance higher than that for the ratio of conductivity to freezing-point depression. In the second series the same relationship holds in only 7 of the 10 comparisons. The differences in both first and second series are generally low. We have no explanation of this result to suggest.

A comparison of the coefficients of correlation for hydrogen-ion concentration with that for specific electrical conductivity indicates that in the first series of determinations the correlation for conductivity is higher than that for hydrogen-ion concentration, whereas in the second series of determinations the reverse is true. This result is substantiated by a comparison of the coefficients for the ratio of conductivity to freezing-point depression with those for hydrogen-ion concentration.

The determination of the environmental (soil or atmospheric) or the internal conditions to which these differences are due presents a problem for future investigation.

SUMMARY AND CONCLUSIONS

While this paper is in substance a contribution to the comparative physiology of Egyptian and Upland cotton, its purpose has been broader. It is one of a series of investigations which have been undertaken with the conviction that the relative capacity for growth and production of different crop plants under special conditions ultimately depends upon intrinsic morphological and physiological differences, and that in our attempts to secure varieties of crop plants which may be successfully grown under particularly stringent environmental conditions (such as those of extremes of temperature, or of aridity of the soil or atmosphere, or of the concentration or reaction of the soil solution) we will in the long run make the most rapid and certain progress by determining the particular variables which fit the plant for growth under these conditions. With a working knowledge of these variables, based on investigations of native vegetations and of crop plants, it will be possible to select varieties which seem most suitable for growth under peculiar environmental conditions.

The present investigation has been limited to osmotic concentration, specific electrical conductivity and acidity in terms of hydrogen-ion concentration in the leaf-tissue fluids of Pima Egyptian and of Meade and Acala Upland cottons and of those of the F_1 hybrid between Pima and Meade cotton as grown under irrigation in southern Arizona. This has been in part because the technique for work with these variables is in a more satisfactory state of development than that for others which may be of importance. The limitation has, however, been primarily because investigations on natural vegetation have indicated a close relationship between the first two of these variables and the dryness and salinity of the substratum.

The statistical constants for sap properties are based on a large number of determinations on plants grown in 1921 on subplots at the Cooperative Testing Station, Sacaton, Ariz. The determinations were made in two series, the first based on collections of tissue made from August 6 to August 16; the second on samples of tissue gathered from August 19 to

August 27. The results for the two series of determinations differ slightly but are mutually confirmatory, and substantiate those of preliminary determinations made in 1920. They establish the following differences between the tissue fluids of the Egyptian and the Upland type of cotton.

The osmotic concentration of the leaf-tissue fluids is higher in Egyptian than in Upland cotton. Thus, in the first series of determinations, the freezing-point depressions of the Egyptian plants grown from self-fertilized seed average 1.404 as compared with 1.353 in the associated Meade plants grown from self-fertilized seed; the Pima plants grown from bulk seed average 1.357 as compared with 1.352 in the associated Meade plants grown from bulk seed; the Pima plants grown from bulk seed average 1.340 as compared with 1.300 in the associated Acala plants grown from bulk seed. In the second series of determinations the average for Pima plants from self-fertilized seed is 1.298 as compared with 1.212 in the Meade plants from self-fertilized seed; the average for Pima plants from bulk seed is 1.257 as compared with 1.201 for Meade plants from bulk seed; the average for Pima plants grown in association with Acala is 1.280 as compared with 1.182 in the Acala. While the differences are not large, varying from less than 1 per cent to slightly more than 8 per cent, they are in the main far larger than their probable errors and are consistent throughout in indicating a higher osmotic concentration in the tissue fluids of the Egyptian cotton. The differences are greater in the second series than in the first.

While these differences are small as compared with those which have been demonstrated between the native plant species of humid and those of arid regions, they may be of significance in the growth of the plants. Critical experiments to determine whether this is the case still remain to be made.

The electrical conductivity of the leaf-tissue fluids of Egyptian cotton is significantly higher than that of either of the Upland cottons compared. This result is found consistently in each of the series investigated. The differences range from 2.93 to 3.77 per cent in the first series and from 8.19 to 9.22 per cent in the second series. The difference between the two cottons is greater in the collections made at the later date, notwithstanding the fact that the actual values of conductivity are lower.

The higher values of electrical conductivity indicate that the Pima Egyptian cotton is capable of taking up from the soil and retaining in solution in the tissue fluids larger quantities of conducting electrolytes than the Upland cottons considered.

The ratio of specific electrical conductivity, κ, to freezing-point depression, Δ, is in general somewhat higher in Egyptian than in Upland cotton. The differences are not, however, large.

This result suggests that the tissue fluids of the Egyptian cotton contain relatively, as well as absolutely, larger quantities of solutes capable of carrying the electric current than those of Upland cotton.

The reaction of the expressed tissue fluids is acid in both types of cotton. The values of P_H are lower in Egyptian than in the Upland type. Thus acidity, as measured in terms of hydrogen-ion concentration, is greater in the Egyptian than in the Upland. In the first series of determinations the value of P_H is 5.245 in the Pima as compared with 5.346 in the associated Meade plants grown from self-fertilized seed; 5.269 in the Pima as compared with 5.396 in the associated plants grown from bulk

Meade seed; 5.257 in the Pima as compared with 5.302 in the associated Acala plants. In the second series of determinations the differences are of about the same order.

A comparison of the sap properties of the F_1 hybrids between Pima and Meade (P × M) with those of the two parents gives the following results:

The osmotic concentration of the tissue fluids of the hybrid is lower than that of either of the parent types.

When the hybrid is compared with parental cultures grown from self-fertilized seed, the hybrid has an osmotic concentration 3.87 per cent lower than that of the Meade parent and 7.36 per cent lower than that of the Pima parent in the first series of determinations, and 6.18 per cent lower than that of the Meade parent and 12.4 per cent lower than that of the Pima parent in the second series of determinations.

When the hybrid is compared with the parental forms grown from bulk seed, the hybrid has an osmotic concentration 4.34 per cent lower than that of the Meade parent and 4.75 per cent lower than that of the Pima parent in the first series of determinations, and 8.18 per cent lower than that of the Meade parent and 12.3 per cent lower than that of the Pima parent in the second series of determinations.

Since the Upland cotton has a lower osmotic concentration than the Egyptian, the difference between the hybrid and the Upland type is of necessity smaller than that between the hybrid and the Egyptian type. The difference between the hybrid and the parent forms is greater in the samples taken later in the season.

The specific electrical conductivity of the hybrid is in all cases lower than that of either of the parent types. In the first series of determinations the conductivity of the hybrid tissue fluids was 4.35 per cent lower than that of the Meade parent and 7.84 per cent lower than that of the Pima parent when the two parents were grown from self-fertilized seed. The hybrid was 1.53 per cent lower than the Meade parent and 4.39 per cent lower than the Pima parent when the parent forms were grown from bulk seed. In the second series of determinations the percentage differences range from 0.33 per cent in comparison with the self-fertilized Meade parent to 9.11 per cent in comparison with the Pima parent from bulk seed.

In general, the differences between the parent and the hybrid forms are greater in the determinations made later in the season.

Since the conductivity is lower in the Upland than in the Egyptian type, the difference between the constant for the hybrid and that for the Upland parent form is smaller than that between the hybrid and the Egyptian parent type.

The ratio of specific electrical conductivity to freezing-point depression, κ/Δ, is generally but not invariably higher in the hybrid than in the parent forms. In the first series of determinations the ratios are sometimes higher in the hybrid and sometimes higher in the parents. In the second (later) series, the hybrid gave a ratio from 3.7 to 7.1 per cent higher than the parent forms. The differences are, therefore, larger and more clearly significant in comparison with their probable errors in the later than in the earlier series of determinations.

The hydrogen-ion concentration of the hybrid is higher than that of the Egyptian parent, but lower than that of the Upland parent. Thus the hybrid tissue fluids are less acid than those of the Pima parent, but

more acid than those of the Meade parent. In short, the hybrid is intermediate in acidity between the two parental forms.

The results for hydrogen-ion concentration are of particular interest since in respect to this physical constant the behavior of the hybrid is quite different from that in respect to osmotic concentration and specific electrical conductivity, both of which show values for the hybrid which are distinctly lower than those of either of the parent forms.

Hydrogen-ion concentration is therefore in full agreement with the great majority of morphological characters (other than size) with respect to which the hybrid is intermediate between the two parent forms.

The foregoing statements summarize the results as far as a comparison of the two cottons and their F_1 hybrid is concerned. We now have to consider certain outstanding features of these series of constants as a whole.

Conspicuous among these general features is the fact that practically all the constants differ in the two series of determinations, made earlier and later in the month of August.

Both osmotic concentration as measured by the depression of the freezing point, Δ, and electrical conductivity, κ, are lower in the second series of determinations than in the first, in the case of both hybrids and parents. The differences are relatively large, and clearly significant. The ratio of specific electrical conductivity to freezing-point depression, κ/Δ, is on the average lower in the second series of determinations made on the parent forms, but higher in the second series made on the hybrids. The difference between the first and second series of determinations made on the hybrids is much larger than that between the first and second series made on the parent forms. The values of P_H, measuring acidity in terms of hydrogen-ion concentration, are uniformly higher in the second than in the first series of determinations. Since higher values of P_H indicate lower acidities it is clear that the concentration of the hydrogen ion is also lower in the second series.

Thus the concentrations of total solutes (molecules and ions) of all ionized solutes and of the hydrogen ion are lower in the second than in the first series. The explanation of these changes must await further investigation.

In its bearing on the problem of the differentiation of the two types of cotton it is perhaps significant that the second series, while showing absolutely lower values of osmotic concentration, specific electrical conductivity, and concentration of the hydrogen ion, shows uniformly a greater differentiation with respect to these variables. Thus the differentiation between the two types increases with the advance of the season.

The direct and the cross correlations between the homologous samples of the first and second series of determinations have been discussed. It is shown that the relationships for the Egyptian is very different from that for the Upland and hybrid plants. The explanation of these differences must await further investigation.

There is a significant positive correlation between the members of the same duplet or triplet (the plants or groups of plants of the two or three types immediately associated at the same station of the field). This indicates a large influence of soil salinity, or some other factor of substratum heterogeneity, or an influence of variations in atmospheric conditions during the period throughout which the samples were taken.

In these correlations between the plants of the same duplet or triplet, the coefficients for osmotic concentration are higher than those for specific electrical conductivity, or for the ratio of specific electrical conductivity to freezing-point depression. They are in practically every instance higher than those for hydrogen-ion concentration. Thus the concentration of total solutes seems to be more influenced by the environmental conditions than the concentration of all electrolytes or of the hydrogen-ion.

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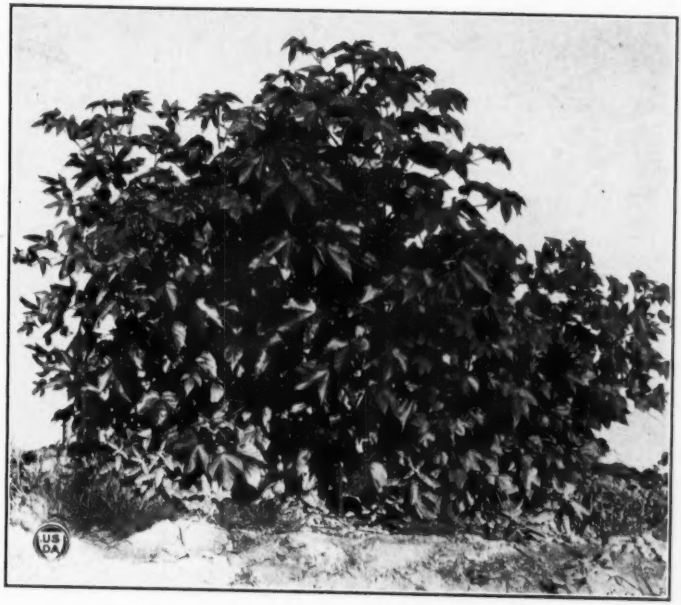
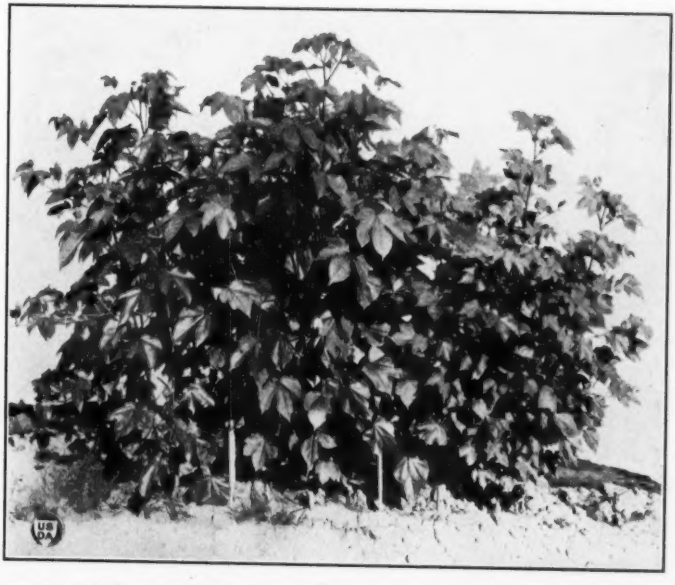
PLATE 1

Typical triplets of Pima Egyptian, Meade Upland, and F_1 hybrid between Pima and Meade cotton, showing in each figure a plant of Pima at the left and a plant of Meade at the right of the F_1 hybrid, which occupies the center of the group.